EFFECTS OF SOIL AMENDMENTS ON GROWTH PERFORMANCE, PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF *Melastoma malabathricum* L. GROWN ON TROPICAL ACIDIC SOIL

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INSTITUTE OF BIOLOGICAL SCIENCES
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EFFECTS OF SOIL AMENDMENTS ON GROWTH PERFORMANCE, PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF *Melastoma malabathricum* L. GROWN ON TROPICAL ACIDIC SOIL

ABSTRACT

Soil acidity, primarily caused by the leaching of basic cation, acid deposition and the decomposition of organic matter led to a decrease in soil pH. Growth under an abiotic stress environment, particularly on low soil pH, typically decreases the growth performance of a plant. Among the plant species, *Melastoma malabathricum* L., or sendudok has adapted to thrive in tropical acidic soil conditions and it successfully stabilizes the problematic soil, prevents landslides, and enhances the aesthetic values of the landscape. However, its adaptation to low pH and fertility resulted in a lower growth rate and shallow root profile. To ameliorate this downside, soil amendments can be applied. Organic wastes from the food waste and palm oil industries can be turned into compost and biochar and used as soil amendments to enhance the soil quality and growth performance of plants grown on infertile acidic soil. Thus, this study aimed to elucidate the effect of soil amendments on growth and nutrient uptake through a six-month study period at the Rimba Ilmu glasshouse, Universiti Malaya. Eight treatments were evaluated in this study; control (Q), palm kernel biochar (B), a combination of liming and palm kernel biochar (BL), a combination of food waste compost and palm kernel biochar (BC), a combination of liming, food waste compost and palm kernel biochar (BCL), food waste compost (C), liming (L) and a combination of liming and compost (CL). The best two treatments, PK biochar (B) and FW compost (C) were further evaluated in terms of plant's cellular antioxidant, oxidative stress indicators and the distribution of metabolites. Treatment with B showed the best growth in terms of *M. malabathricum* L. height, stem diameter, leaf area index, root length and root length density. In physiological performance, treatment C recorded a significantly higher rate of photosynthesis and

relative chlorophyll content by 16% and 154%, respectively in comparison to the control. For soil analysis, both treatments B and C showed comparable results. Treatment C and B also showed improved H_2O_2 level in all parts of the plant. The enzymatic antioxidants activities and non-enzymatic antioxidants were also higher in treatment C. In addition, the same treatment exhibited the highest total anthocyanin content (leaves; 36.1 x 10^{-2} ± 0.034 mg/g DW and root extract; 8.9 x 10^{-2} ± 0.020 mg/g DW), total phenolic content (stem extract; 4930.956 ± 16.025 mg GAE/g DE) and total flavonoid content (stem extract; 209.984 ± 0.572 mg QE/g DE). The *M. malabathricum* L. plants that were grown without soil amendment, (Q) recorded the highest number of metabolites (leaves; 57 and root extract; 40) followed by treatment C (leaves; 46 and root extract; 31) and treatment B (leaves; 26 and root extract; 31). The findings of this study indicate that the PK biochar, followed by FW compost substantially enhanced the growth and physiological characteristics of *M. malabathricum* L., resulting in taller plants, larger stem diameters, increased root length and density, and enhanced chlorophyll content and photosynthesis. The soil analysis revealed that the use of PK biochar and FW compost retained nutrients, enhanced soil pH, and increased water retention, but lime had a higher nutrient absorption rate. Concerning oxidative stress indicators, the treatment with FW compost and PK biochar enhanced the plant's antioxidant defense system against oxidative damage by increasing the activities of antioxidant enzymes such as SOD and APX, as well as the synthesis of antioxidant metabolites such as chlorophylls, carotenoid, ascorbic acid, anthocyanin, phenolic and flavonoid compounds, compared to control plants. These demonstrate the potential of both treatments as soil amendments to not only improve soil properties, but also improve *M. malabathricum* L. plants' tolerance towards abiotic stress (soil acidity). In conclusion, this research demonstrates the positive impacts of food waste compost, followed by palm kernel biochar as soil supplements on the growth performance, physiological features, and biochemical properties of *M. malabathricum* L.

when cultivated in acidic soil. The effect of these discoveries include boosting plant growth in environments where abiotic stress is present and improving the soil fertility.

Keywords: *Melastoma malabathricum* L., food waste compost, palm kernel biochar, acidic soil

PENGARUH PERUBAHAN TANAH TERHADAP KEBERHASILAN PERTUMBUHAN, SIFAT FISIOLOGI DAN BIOKIMIA *Melastoma malabathricum L.* YANG DITANAM DI TANAH TROPIKA BERASID

ABSTRAK

Keasidan tanah, terutamanya disebabkan oleh pelunturan kation asas, pemendapan asid dan penguraian bahan organik menyebabkan penurunan pH tanah. Pertumbuhan di bawah persekitaran tekanan abiotik, terutamanya pada pH tanah rendah, biasanya mengurangkan prestasi pertumbuhan tumbuhan. Di antara spesies tumbuhan, *Melastoma malabathricum* L., atau sendudok telah menyesuaikan diri untuk berkembang dalam keadaan tanah berasid tropika dan ia berjaya menstabilkan tanah yang bermasalah, menghalang tanah runtuh, dan meningkatkan nilai estetik landskap. Walau bagaimanapun, penyesuaiannya terhadap pH dan kesuburan yang rendah menyebabkan kadar pertumbuhan yang lebih rendah dan profil akar cetek. Untuk memperbaiki kelemahan ini, pembaikan tanah boleh digunakan. Sisa organik daripada industri sisa makanan dan minyak sawit boleh dijadikan kompos dan biochar dan digunakan sebagai pembaikan tanah untuk meningkatkan kualiti tanah dan prestasi pertumbuhan tumbuhan yang ditanam di tanah berasid tidak subur. Oleh itu, kajian ini bertujuan untuk menjelaskan kesan pembaikan tanah terhadap pertumbuhan dan pengambilan nutrien melalui tempoh kajian selama enam bulan di rumah kaca Rimba Ilmu, Universiti Malaya. Lapan rawatan telah dinilai dalam kajian ini; kawalan (Q), biochar isirung sawit (B), kombinasi kapur dan biochar isirung sawit (BL), kombinasi kompos sisa makanan dan biochar isirung sawit (BC), kombinasi kapur, kompos sisa makanan dan biochar isirung sawit (BCL), kompos sisa makanan (C), kapur (L) dan kombinasi kapur dan kompos (CL). Dua rawatan terbaik, biochar isirung sawit (B) dan kompos sisa makanan (C) telah dinilai lebih lanjut dari segi antioksidan selular tumbuhan, penunjuk tekanan oksidan dan agihan metabolit. Rawatan dengan B menunjukkan pertumbuhan terbaik dari segi ketinggian *M. malabathricum* L., diameter

batang, indeks keluasan daun, panjang akar dan ketumpatan panjang akar. Dalam prestasi fisiologi, rawatan C mencatatkan kadar fotosintesis yang jauh lebih tinggi dan kandungan klorofil relatif sebanyak 16% dan 154%, masing-masing berbanding dengan kawalan. Untuk analisis tanah, kedua-dua rawatan B dan C menunjukkan hasil yang setanding. Rawatan C dan B juga menunjukkan tahap H_2O_2 yang lebih baik di semua bahagian tumbuhan. Aktiviti antioksidan enzim dan antioksidan bukan enzim juga lebih tinggi dalam rawatan C. Di samping itu, rawatan yang sama menunjukkan jumlah kandungan antosianin tertinggi (daun; $36.1 \times 10^{-2} \pm 0.034$ mg/g DW dan ekstrak akar; $8.9 \times 10^{-2} \pm 0.020$ mg /g DW), jumlah kandungan fenolik (ekstrak batang; 4930.956 ± 16.025 mg GAE / g DE) dan jumlah kandungan flavonoid (ekstrak batang; 209.984 ± 0.572 mg QE/g DE). Tumbuhan *M. malabathricum* L. yang ditanam tanpa pembaikan tanah, (Q) mencatatkan bilangan metabolit tertinggi (daun; 57 dan ekstrak akar; 40) diikuti dengan rawatan C (daun; 46 dan ekstrak akar; 31) dan rawatan B (daun; 26 dan ekstrak akar; 31). Penemuan kajian ini menunjukkan bahawa biochar isirung sawit diikuti oleh kompos sisa makanan dengan ketara meningkatkan ciri pertumbuhan dan fisiologi *M. malabathricum* L., mengakibatkan tumbuh-tumbuhan yang lebih tinggi, diameter batang yang lebih besar, panjang dan ketumpatan akar yang meningkat, dan meningkatkan kandungan klorofil dan fotosintesis. Analisis tanah mendedahkan bahawa penggunaan biochar isirung sawit dan kompos sisa makanan mengekalkan nutrien, pH tanah yang dipertingkatkan, dan peningkatan retensi air, tetapi kapur mempunyai kadar penyerapan nutrien yang lebih tinggi. Mengenai penunjuk tekanan oksidan, rawatan dengan kompos sisa makanan dan biochar isirung sawit meningkatkan sistem pertahanan antioksidan tumbuhan melawan kerosakan oksidatif dengan peningkatan aktiviti enzim antioksidan seperti SOD dan APX, serta sintesis metabolit antioksidan seperti klorofil, karotenoid, asid askorbik, antosianin, fenolik dan sebatian flavonoid, berbanding dengan tumbuhan kawalan. Ini menunjukkan potensi kedua-dua rawatan sebagai pembaikan tanah bukan

sahaja meningkatkan sifat tanah, tetapi juga meningkatkan toleransi tumbuhan *M. malabathricum* L. terhadap tekanan abiotik (keasidan tanah). Kesimpulannya, penyelidikan ini menunjukkan kesan positif kompos sisa makanan, diikuti oleh biochar isirung sawit sebagai makanan tambahan tanah terhadap prestasi pertumbuhan, ciri fisiologi, dan sifat biokimia *M. malabathricum* L. apabila ditanam di tanah berasid. Kesan penemuan ini termasuk meningkatkan pertumbuhan tumbuhan dalam persekitaran di mana tekanan abiotik hadir dan meningkatkan kesuburan tanah.

Kata kunci: *Melastoma malabathricum* L., kompos sisa makanan, biochar isirung sawit, tanah asid

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LIST OF ABBREVIATIONS

$°C$	:	Degree Celsius
%	:	Percentage
>	:	More than
<	:	Less than
μL	:	Microliter
ABA		Abscisic acid
$AlCl3.6H_2O$	:	Aluminium chloride
ANOVA	:	One-way analysis of variance
cm	:	Centimeter
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DMRT	:	Duncan's multiple range test
FCR	:	Folin–Ciocalteu reagent
Fe^{2+}	:	Ferrous
Fe^{3+}	:	Ferric
$FeSO^4$	:	Iron (II) sulfate
FRAP	:	ferric reducing power
IAA		Indole-3-acetic acid
IBA		Indole-3-butyric acid
g	:	Gram
g GAE/g DE	:	Gram of gallic acid equivalents/gram of dry extract
g QE/g DE	:	Gram of quercetin equivalents/g of dry extract
h		hour
LC-MS	:	Liquid chromatography – mass spectrometry

mg/L	:	Milligram per liter
mg/mL	:	Milligram per milliliter
min		minutes
mM	:	millimolar
N	:	Nitrogen
NMR	:	Nuclear magnetic resonance
ROS	:	Reactive oxygen species
SE	:	Standard error
SPSS	:	Statistical Package for the Social Sciences
TAC	:	Total anthocyanins content
TCC		Total compound chromatogram
TFC	:	Total flavonoid content
TPC	:	Total phenolic content
UV	:	Ultraviolet
v/v	:	Volume per volume

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CHAPTER 1: INTRODUCTION

1.1 Research Background

The acidification of soil is a prevalent problem throughout the world, including Malaysia. Approximately 70% of the soil in Malaysia are Ultisols and Oxisols, which are highly weathered, acidic and infertile for crop production (Shamshuddin et al., 2010). During geological evolution, especially in high rainfall areas, bases are relatively easy to leach from soils, thus leaving them acidic. In tropical regions, a lack of buffer capacity and low clay activities, which results in soil acidity, caused the soil to become arid and barren. Infertile soil caused by soil acidification has become one of the primary constraints to plant growth and development. The productivity of a plant and soil fertility are adversely affected by acidic soil (Osuji et al., 2007). The shallow root produced with the poor performances of the plants has ramifications on nutrient acquisition (Opala, 2011), plant establishment and survival (Fekadu et al., 2018).

Melastoma malabathricum L. has the capacity to survive in extreme soil conditions, including acidic soil, thus it is one of the most suitable plant for phytoremediation (Dorairaj et al., 2020). In addition, *M. malabathricum* L. has excellent physiological performance attributed to its high growth rate (Halim & Normaniza, 2015). Having the M-type root architecture, this feature is common among herbs plants and most of the root branches grow in various directions (Styczen et al., 2003). This is due to their shallow roots in which most of their main root grows abundantly under stump or base (Ali, 2010). Besides that, *M. malabathricum* L. has higher water use efficiency (WUE) and a more extended root profile on acidic soil. Significant morphological and physiological changes have been reported in *M. malabathricum* L. in response to severe acidic soil pH (Dorairaj et al.,2020). These changes include the improved photosynthetic rate, transpiration rate,

leaf area index (LAI) and root system. Besides that, the work of Halim & Normaniza (2015) extends the idea that *M. malabathricum* L. works best on the slope in terms of its stomatal conductance, photosynthetic and transpiration rates, as well as high WUE. In response to acidic soil, the growth of shoots and roots was observed to be reduced, thus rendering this a challenging task as the slope surface requires larger canopy coverage and excellent root anchorage. Additionally, it was demonstrated by Saifuddin et al. (2016) that the planting of this species resulted in a significant increment of soil pH and has the capacity to restore the slope's acidic condition. Therefore, it would be essential to cultivate acid-tolerant plants to rehabilitate the acidic soil.

To mitigate soil acidity, various approaches have been identified by Pauw (1994) including toxicity alleviation through the use of liming material and fertilizer; to boost soil organic matter levels and to grow acid tolerant plants and cultivars. Among these strategies, organic wastes are recommended in recent studies as soil amendments greatly influence the cation exchange capacity (CEC), soil pH, plant productivity, soil aggregate and stability, bulk density, infiltration rate and water holding capacity (Lee et al., 2019; Sayara et al., 2020; Yu et al., 2019). The circular economy can also be supported by the use of waste-derived soil amendments because it maximizes the use of resources including waste generated by various industries (Sherwood, 2020). In this study, agricultural waste and municipal solid waste were used to examine and compare the growth performance, physiological and biochemical properties of *M. malabathricum* L. under acidic conditions.

1.2 Problem Statement

Soil acidification has a detrimental impact on soil fertility, biological activities and plant productivity (Soriano, 2014). A lower soil pH can have a toxic effect on a plant's

morphology and physiology, which resulted in stunted plant growth and, eventually, a lower crop yield. This abiotic stress can lead to the accumulation of primary and secondary metabolites from the activated multigene responses (Rodziewicz et al., 2014) and the development of effective defenses against highly reactive oxygen species (ROS). ROS has dual functions in plants; at low concentrations, it serves as a signal for stress tolerance, whereas at higher concentrations, it can cause cellular damage and programmed cell death (Foyer, 2018). To maintain the equilibrium between the production and removal of ROS, plants have evolved scavenging activities composed of highly effective enzymatic and non-enzymatic antioxidant defense systems (Moradbeygi et al., 2020). The ROS brought on by abiotic stresses has also been essential for the survival of plants (Nakabayashi et al., 2015).

There were several studies that investigated the influence of abiotic stress on vegetation traits. However, studies on the associating effect of acidic soil conditions on plant physiological performance, which include the synthesis of specialized metabolites are lacking in the main literature. Acidic soil tolerance varies widely between plants. The most effective way to manage soil acidity is to breed tolerant plant (Bian et al., 2013). Amongst the plant species, *Melastoma malabathricum* L. has gained significant research interest in tropical countries such as Malaysia due to its maximum tolerance for acidic soil, as measured by plant morphological parameters and photosynthetic capacity (Dorairaj et al., 2020). Existing literature identifies a research gap regarding the effect of acidic soil on plant growth, specifically the observed retardation of growth rate and shoot biomass, necessitating further investigation to better understand the underlying mechanisms and to develop potential mitigation strategies.

The common practice to curb this issue is by increasing the soil pH. The application of lime has been the most common practice for many years. However, excessive liming can inhibit plant growth and reduce the availability of some micronutrients that is essential for plant growth (Olego et al., 2021; Yan et al., 2021). Furthermore, liming can be expensive over time, particularly if it is done on a large scale (Warner et al., 2023). Organic amendments such as compost and biochar can be used as an alternative to lime for improving soil fertility. These amendments are a useful tool for enhancing soil fertility through improved soil structure, water retention and nutrient levels (Agegnehu et al., 2016). Additionally, organic amendments help in preserving a more stable pH level in the soil, which is necessary for optimum plant development by improving soil biophysical and chemical properties, increasing crop yield and enhancing the long-term sustainability of the production system. Furthermore, it can lessen the need for synthetic fertilizers and other inputs, thus making soil health maintenance a more sustainable and affordable option in the long run. Increased soil pH, EC and CEC, as well as a decrease in exchangeable acidity, demonstrate the effectiveness of biochar in reducing acidity (Chintala et al., 2014). High porosity in biochar encourages microbial activity and increases soil pH due to its alkaline chemical characteristics, improving nutrient retention and overall soil fertility (Mosharrof et al., 2021; Tomczyk et al., 2020). A study by Agegnehu et al., (2016) has proven that applying organic amendments encouraged the growth and productivity of maize with a significantly increased grain yield (10- 29%) and total biomass (9-18%) compared to the control plant.

Many amendments were introduced in the literature, however, most lack further research on which ones are the most effective in acidic conditions in terms of increasing soil fertility and inducing plant growth. Additionally, there is a need to investigate the relationship between ROS-mediated metabolite distribution and their role in enhancing

soil fertility and promoting plant growth. The practical use of organic amendments towards problematic soil is expected to grow dramatically to improve plant survival in the current environmental changes. Nevertheless, it is unclear whether the mechanisms involved in organic amendments from palm kernel biochar and food waste compost-induced amelioration of acidic stress are differ from those of other abiotic stresses. Hence, this study is required to provide evidence of underlying similarities and differences in plant development, the physiological responses and the induction of secondary metabolites that may be connected with its influence on ROS and oxidative responses.

1.3 Significance of the Study

The application of organic amendments has received increasing attention and promising green materials for the plant growth and productivity. Currently, there is no evidence of the application of organic amendments toward *M. malabathricum* L. Hence, this study was conducted to investigate the effect of organic amendments on soil quality and the nutrients of *M. malabathricum* L., including its growth performance. The outcome of this study may contribute to the knowledge on the growth and physiological characteristics of *M. malabathricum* L. thriving under low pH conditions. Understanding the biochemical changes induced by organic amendment application, which can shed light on the mechanisms underlying plant stress tolerance, will also add to the database of acidic stress tolerant plants that have been specifically tailored to overcome problematic soils. Thus, a deeper understanding of the distribution of the metabolites in shoots and roots together with the biological activity of *M. malabathricum* L. can be achieved using amendments as an alternative nutrient supplement for the soil in the future. The findings from this study will have far-reaching implications for sustainable crop production under abiotic stress conditions. In regions prone to abiotic stress, the acquired knowledge led to innovative strategies for enhancing plant resilience and reducing crop losses.

Furthermore, using organic amendments as soil ameliorator can promote organic waste recycling, reducing reliance on chemical fertilizers and contribute to environmental sustainability.

1.4 Objectives of the Study

The objectives of this research are as follows:

1. To investigate the effect of soil amendments on the growth and nutrient uptake of *M. malabathricum* L. grown on tropical acidic soil conditions.
2. To analyze the effect of soil amendments on physiological characteristics of *M. malabathricum* L. in acidic conditions.
3. To analyze plant cellular antioxidant and oxidative stress indicators affected by soil amendment in acidic conditions.
4. To analyze the differential distribution of auxin in the shoot and root of *M. malabathricum* L. treated by soil amendment in acidic conditions.

1.5 Scope of the Study

The scope of this study is to investigate the effect of organic amendments focusing on palm kernel biochar and food waste compost on the growth performance, and physiological and biochemical properties of *M. malabathricum* L. under abiotic stress conditions. Chapter 1 introduces the research topic, outlining the background, problem statement and research objectives. The significance of the study is also emphasized to highlight the relevance and potential contributions of the research. In Chapter 2, a comprehensive literature review is conducted, exploring existing knowledge on the impact of organic amendments on plant growth, as well as its effects on physiological and biochemical aspects in the context of abiotic stress. Chapter 3 presents the methodology

employed including the selection of plant species, abiotic stress factors, soil amendments preparation, experimental setup and data collection methods. Chapter 4 presents the results obtained from the study, showcasing the growth performance parameters and changes in the physiological and biochemical properties of the plants. Chapter 5 engages in a thorough discussion of the results, interpreting and analyzing the findings in relation to existing literature. Finally, Chapter 6 presents the conclusion, summarizing the study's key findings, their implications and recommendations based on the research outcomes. The limitations of the study are acknowledged, and suggestions for future research are provided.

CHAPTER 2: LITERATURE REVIEW

2.1 Acidic Soil and Plant Growth

Soil plays a pivotal role in maintaining biodiversity. This is due to the ability of the soils to restrain the hydrological, biogeochemical and nutrient cycle (Smith et al., 2015). Soil sustainability in this globalized world of the 21st century greatly depends on the management practice by farmers, foresters, land planners and politicians. Some progress has been made, however, extensive land and soil degradation still occur all over the world and fertile soil resources are rapidly depleted (Keesstra et al., 2016). The soil system is greatly affected by the misuse of soil resources (Lal, 1987), which can cause the degradation of soil structure such as accelerated erosion, depletion of soil organic carbon pool, loss in biodiversity, loss of soil fertility and element imbalance, salinization and acidification (Lal, 2015; Masto et al., 2015).

According to Sumner et al. (2003), acid soils are divided into two types: naturally occurring acid soils and anthropogenically derived acid soils. Naturally occurring acid soils is due to intensive weathering and acid sulfate soils whilst anthropogenically derived acid soils can be from acid deposition, intensively managed row crop agriculture and pasture systems. Over the years, soil pH of 5.5 or lower has become the most critical constraint for crop production worldwide (Agegnehu et al., 2021). The production of staple food crops, and in particular grain crops, is negatively impacted by acidic soils. Soil acidity is an important factor to be considered due to the lack of buffer capacity and low clay activities. Another factor that promotes soil acidity is the leaching of basic elements such as calcium, magnesium, potassium and sodium from the soil when rains take place (De Vries et al., 1987).

Most soils in Malaysia are acidic, thus it is not suitable for the growth of most plant species. The growth and reproduction of plant species are affected by low pH. Plant growth is hampered by acidic soil in a variety of ways but toxicities have been identified as one of the most prevalent causes (Robson, 2012). Toxicity is mainly associated with the high concentration of aluminium (Al) together with other acidic elements such as manganese (Mn), hydrogen (H) and iron (Fe). At soil pH values of 5 or below, crop yield production becomes lesser as the root growth and function were prohibited due to the formation of Al in the soil solution (Alia et al., 2015). The poor root-soil contact and reduced root length are the main reasons of a slower capacity to thrive in acidic soil (Haling et al., 2010). These effects have implications for uptake of essential nutrient for plant growth. Acidic soil caused the chemical structures and reactions that govern nutrient availability and absorption by plant roots be altered, thus reduced the immobilization of necessary nutrient (Borhannuddin Bhuyan et al., 2019).

Soil without vegetation cover becomes barren and unstable with time. This has caused geographical as well as environmental problems including erosion and landslide. Vegetation provides root reinforcement, soil moisture depletion, buttressing, arching and surcharge (Fan et al., 2008). Nevertheless, soil acidity inhibits the elongation and interconnection of the root system (Caires et al., 1991; Shamshuddin et al., 2013). Plant growth is affected by soil acidity due to poor root systems thus leading to the reduction in nutrient uptake. The root structure serves a crucial role for the plant such as anchoring the plant to the soil, nutrient acquisition and water uptake (Han et al., 2017). Moreover, it has been found that changes in the abiotic factor reduce root elongation and restricts the plant roots to explore the acidic subsoil to absorb water (Yang et al., 2013). Thus, it is necessary to develop a new approach to restore and rehabilitate acidic soils, which can be based on the use of amendments.

Additionally, soil acidity has an impact on photosynthesis, primarily by reducing the leaf area, chlorophyll content, transpiration rate and stomatal conductance. According to a study by He et al. (2011), the rate of photosynthesis and stomatal conductance in *Camellia oleifera* Abel in wild and cultivated varieties grown on acidic soil decreased significantly. Al toxicity is regarded as the key factor limiting plant growth on acidic soil. Some crop species have reported a decrease in leaf chlorophyll due to Al toxicity (Mihailovic et al., 2008; Samad et al., 2020). The decrease in the chlorophyll content can be related to the oxidative stress generated by Al toxicity as reported by Alves et al. (2022). The production of ROS can lead to lipid peroxidation, hence triggering thylakoid membrane damage (Killi et al., 2020). Oxidation of membrane lipid produces reactive by-products, such as malondialdehyde (MDA) and aldehydes, which lead to membrane disruption. The increased in MDA can disrupt membrane-associated proteins and pigments, resulting in impaired energy transfer and altered thylakoid structure (Tewari et al., 2021). To evaluate the tolerance of plants towards acidity stress, the growth or survival of the plants is evaluated, as it reflects the up or downregulation of various physiological and biochemical systems within the plant. Thus, in gaining insight into the mechanisms, it is necessary to cultivate acidic stress-tolerant plants.

2.2 Potential Acidic Tolerance Cultivar: *Melastoma malabathricum* L.

Melastoma malabathricum L. (Plate 2.1) or commonly known as sendudok is an invasive weed species in the Malaysian agrosystem that belongs to the family Melastomataceae. This shrub plant is one of the natural plant resources that has gained attention within the scientific world due to its ethnomedicinal value. This plant has also been reported to grow wild in the Indian Ocean Islands throughout South and South-East Asia, China, Taiwan, Australia and the South Pacific Ocean (Wong, 2008). The taxonomy of *M. malabathricum* L. is outlined as follows (Mybis, 2023):

Table 2.1: The taxonomy of *M. malabathricum* L. (Mybis, 2023)

Taxonomic Level	Sendudok
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Myrtales
Family	Melastomataceae
Genus	*Melastoma* L.
Species	*Melastoma malabathricum* L.

Plate 2.1: Pressed and Dried *Melastoma malabathricum* L.

This plant can grow as small shrubs up to 3 ft in height, even occasionally up to 15 ft (Joffry et al., 2012). The plant can be harvested once its reaches maturity, typically after

a period of six months (JPSM, 2016). Traditional medicine practitioners commonly use this species as a remedy for diarrhea (Bhardwaj et al., 2005), gastric ulcers (Lohézic-Le Dévéhat et al., 2002), inflamed wounds (Zakaria et al., 2006), pox scars (Sharma et al., 2001), diabetes and high blood pressure (Joffry et al., 2012).

Several plant species from different genera and families grown on acidic soils have been investigated and the choice of *M. malabathricum* L. is reasonable in terms of its acidic-tolerant capabilities. Having an M-type root architecture, this plant feature is also common among herbs and the roots mostly branch and grow in various directions (Styczen et al., 2003). Besides that, the M-type roots generally enable the plant to grow extensively in various directions and are beneficial in controlling soil erosion by improving the mechanical and hydrological properties (Aimee et al., 2014), thus making them a potential slope remediator (Saifuddin et al., 2016).

Table 2.2: *M. malabathricum* L.'s root growth pattern and mechanical characteristics (Ali, 2010; Osman et al., 2006; Yen, 1972)

Root pattern	Root type illustration	Type of root system	Root tensile strength (MPa)	Point sheer strength (kPa)	Root length density (km m^{-3})
		M-type	29	110.7 ±11.5	22

While its shallow roots and main roots grow abundantly under stump or base (Ali, 2010), *M. malabathricum* L. also has higher water use efficiency (WUE) and a more extended root profile on acidic soils. Besides that, significant morphological and physiological changes were observed in *M. malabathricum* L. in response to severe acidic

soil pH and these changes include improved photosynthetic rate, transpiration rate, leaf area index (LAI) and root system (Dorairaj et al., 2020). Given its ability to tolerate harsh acidic conditions, the plant has also been known to be a potential Al accumulator (Maejima et al., 2017; Watanabe et al., 2005). However, Watanabe et al. (2006) reported that the application of Fe to *M. malabathricum* L. roots without Al led to oxidative stress symptoms such as lipid peroxidation and lignin deposition. Moreover, Halim and Normaniza (2015) has also extended the idea that *M. malabathricum* L. works best on the slope in terms of its stomatal conductance, photosynthetic and transpiration rates and high WUE.

Numerous secondary metabolites have been discovered in *M. malabathricum* L. such as anthocyanin (Anuar et al., 2013), flavonoids and phenolic contents (Sharma et al., 2011). However, the variation of secondary metabolites in plant materials is influenced by abiotic factors, for example water availability, temperature and soil condition (Akula et al., 2011). Various studies have also been conducted on plants to examine the effect of soil amendments on the soil physicochemical properties, bioactive compounds and antioxidant activities of individual crops, however, little is known about such effects on *M. malabathricum* L. grown in acidic conditions.

2.3 Soil Properties

Soil properties become an important factor affecting plant responses including soil chemical properties (pH, cation exchange capacity (CEC), and sufficient and balanced supply of macronutrients and micronutrients), soil physical properties (depth, soil texture and structure, water-holding capacity and soil organic matter (SOM) content), and soil biological properties (soil microbial biomass, beneficial soil microorganisms and soil fauna) (Delgado et al., 2016). In this regard, the soil properties will dictate the best

practice to enhance soil fertility (Koudahe et al., 2022) because the soil has the capacity to provide chemical, physical and biological requirements for plant growth in terms of productivity, reproduction and quality (as measured in terms of human and animal welfare for plants used as food or fodder), which are specific to plant type, soil type, land use and climatic conditions.

2.3.1 Soil Chemical Properties

Soil chemical properties is defined as the capacity of the soils to provide a suitable chemical and nutritional environment for plants and foraging animals that beneficially support soil physical and biological processes including those involved in nutrient cycling to maximize productivity, reproduction and quality (in terms of human and animal well-being) (Koudahe et al., 2022). The important parameter for soil chemical properties is soil pH. Acidification can enhance the mobility of heavy metals (Shu et al., 2001) and lead to the degradation of a revegetation scheme (Yang et al., 2010). High concentrations of hydrogen ions can also inactivate plant enzyme systems, restricting respiration and root uptake (Kinraide, 2003). Cation exchange capacity refers to the ability of soils to store and exchange cations. CEC is often determined by displacing exchangeable cations (Na, Ca, Mg and K) with another strongly adsorbed cation (Hazelton et al., 2016). Subsequently, this determines the amount of strongly adsorbed cations retained by the soils. Acidic soil lowers the cation exchange capacity by increasing the concentration of hydrogen ions, removing essential cations, and potentially releasing toxic aluminium and manganese ions (Harter, 2007). A low CEC in acidic soil reduces the availability of essential nutrients, impairs nutrient balance, causes pH instability, and lowers water-holding capacity (McCauley et al., 2009). Collectively, these factors inhibit plant growth and can lead to nutrient deficiencies, imbalances, and poor plant health. As evidenced in research, sandy soils and acid soils that have been heavily leached frequently have very

low exchangeable calcium and magnesium levels, thus limiting plant growth (Croker et al., 2004).

2.3.2 Soil Physical Properties

Soil depth, soil texture, soil structure, soil water and soil air are categorized under soil physical properties (Roy et al., 2006). Soil physical properties is defined as the capacity of soils to provide physical conditions that support plant productivity, reproduction and quality (considered in terms of human and animal well-being) without leading to the loss of soil structure or erosion while supporting soil biological and chemical processes (Koudahe et al., 2022). This, in turn, exerts an important influence on potential rooting volume, penetrability of roots, water holding capacity, degree of aeration, living conditions for soil life and nutrient mobility and uptake (Roy et al., 2006). The infertile nature of acidic soil is a result of its physical characteristics. The use of organic soil amendments enhances soil physical properties such as soil porosity and water-holding capacity (Wang et al., 2019). Moreover, organic soil amendments have positive impacts such as significantly decreased soil bulk density and increased soil porosity (Toková et al., 2020).

2.3.3 Soil Biological Properties

Soil biological properties refers to the capacity of organisms living in soils (microorganisms, fauna and roots) to contribute to the nutritional requirements of plants and foraging animals for productivity, reproduction and quality (considered in terms of human and animal well-being) while maintaining biological processes that contribute positively to the physical and chemical states of the soil (Koudahe et al., 2022). Soil biological properties are also inextricably linked to other physical and chemical features of the soil such as aeration, SOM and pH, which influence the activity of numerous

microorganisms in the soil (Delgado et al., 2016).

Previously, the effect of soil pH on soil microbial biomass, microbial activity and microbial community organization has been studied. Many studies have indicated that soil pH and substrate availability are significant determinants in regulating soil microbial activity (Cao et al., 2016; Malik et al., 2018). In addition, the pH of the soil influences the solubility of organic carbon and a decrease in pH results in an increase in Al toxicity. This, in turn, causes changes in the structure of the microbial population as well as changes in the activity of the microbes (Andersson et al., 2001).

2.4 Classification of Plants and Soil Nutrients

Plants use a variety of mineral nutrients from the soil or other growth mediums for metabolism and growth. These elements are essential because a nutrient deficiency inhibits plant metabolism and growth, whilst excessive quantities are toxic to the plant. Nutrients added via fertilizers, composts and biochars can have both good and harmful effects on the ecosystem depending on how these inputs are managed (Singh et al., 2015). Efficient nutrient usage ensures that yields exceed those achieved with inherent soil fertility by correcting either an overall nutrient deficiency or a nutrient imbalance (Davidson et al., 2012). However, using a low amount of inputs creates additional strain on the soil nutrient sources that results in excessive soil nutrient mining and soil fertility depletion, which ultimately culminates in land degradation (Alemu, 2015). There are 18 different nutritional components that are necessary for plant growth (Bhaduri et al., 2014). In general, plant nutrients are classified into two categories: macronutrients and micronutrients.

According to Mitra (2017), the macronutrient is further classified into structural elements (C, H and O), primary nutrients (N, P and K) and secondary nutrients (S, Ca and Mg), while micronutrients are Zn, Fe, Mn, Cu, B, Mo, Cl^-, Co and Ni. It has been shown that the majority of plant nutrients are at their most readily available to plants within the pH range of 6.5 to 7.5, and it has also been found that this pH range is generally quite compatible to the growth of plant roots (Figure 2.1). N, P and S are important plant nutrients that appear to be directly affected by soil pH less than many other plant nutrients, although they still are to some extent. Nevertheless, phosphorus (P) is immediately impacted by these changes. Magnesium and calcium are the most often encountered alkaline earth metals (Teir et al., 2006). As the pH of the soil rises, there is typically a reduction in the amounts of the micronutrients such as manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and boron (B) that are available. When the soil pH is over 7.5, most of the micronutrients become less available compared to when the pH is between 6.5 and 6.8. In fact, the micronutrients are most readily available when the pH is between 6.5 and 6.8. The one notable exception to this rule is molybdenum (Mo), which seems to be less available when the pH level is more acidic and more available when the pH level is more alkaline (Gradziel, 2017).

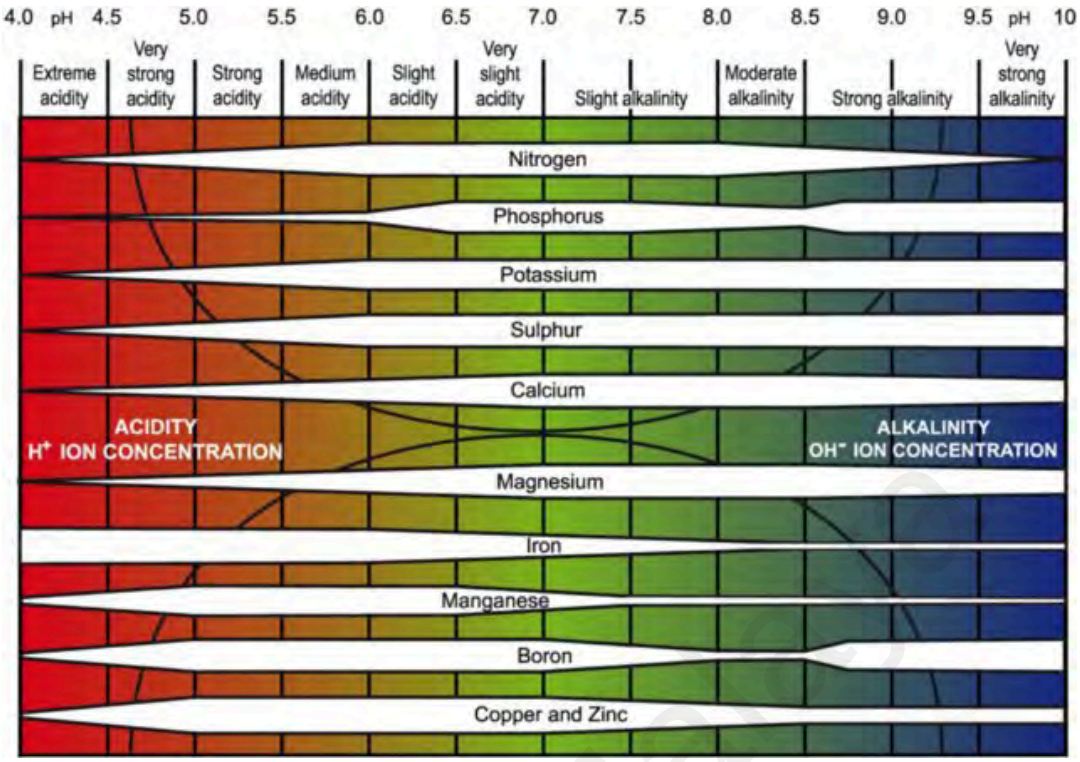


Figure 2.1: Nutrient availability chart based on pH fluctuation (Gradziel, 2017)

2.4.1 Soil Factors Affecting Nutrient Availability

The level of soil pH is a crucial component that determines the availability of macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) that are needed for plant growth. The changes in soil pH have a significant impact on the N content that is available for plant absorption, the availability of soluble P and the availability of K which can either enhance or limit its uptake by plants.

Due to its effect on nitrogen availability, the changes in soil pH can have a substantial impact on plant growth and development. In acidic soils, N is most frequently present as ammonium (NH_4^+), a positively charged ion. This type of nitrogen is more accessible to plants because it is less susceptible to leaching loss than the negatively charged nitrate (NO_3^-) form (Pal et al., 2020). This can result in improved plant growth and yield in acidic soils since plants are able to absorb and utilize more N for vital functions such as photosynthesis and protein synthesis. Nevertheless, soil acidity can also hinder plant

growth in terms of available nitrogen. In extremely acidic soils, the concentration of free hydrogen ions (H^+) can grow excessively high, rendering the soil poisonous to plants, which can inhibit root development and diminish the plant's overall health and output (Miller et al., 1989). Furthermore, in severely acidic soils, the availability of ammonium nitrogen can be limited due to the displacement of positively charged ammonium ions from soil particles by excess hydrogen ions (He et al., 2012). As a result, plants may exhibit stunted growth and decreased yields due to nitrogen shortages. Consequently, even though soil acidity might increase nitrogen availability, it is essential to maintain an optimum soil pH level to avoid deleterious impacts on plant growth.

The effects of soil acidity on the availability of phosphorus can have a substantial effect on plant growth and development. Phosphorus is more readily available and soluble in acidic soils. This is because acidic conditions can trigger the disintegration of soil minerals and organic matter thus releasing bound phosphorus into the soil solution. Yet, high soil acidity can also reduce the availability of phosphorus to plants as the element can become chemically linked to soil particles as insoluble compounds (Roem et al., 2000). This can result in phosphorus deficiency, which can cause stunted growth, poor root development and a reduction in production (Uchida, 2000). Furthermore, the impact of soil acidity on phosphorus availability may have significant ramifications for the entire ecosystem. When excessive amounts of phosphorus are present in the soil, it can leach into the neighboring water sources and create eutrophication, a process in which surplus nutrients promote the growth of algae and other aquatic plants (Bennett et al., 2001). This can lead to dissolved oxygen depletion in the water, which is detrimental to fish and other aquatic life. To support healthy plant development and ecosystem function, it is essential to maintain a balance between soil acidity and phosphorus availability.

Potassium is a vital nutrient for plant growth and plays a crucial part in an extensive array of physiological functions such as water intake, photosynthesis and stress tolerance. Potassium may be less available in acidic soils due to the predominance of hydrogen ions (H^+), which can displace potassium ions (K^+) from soil particles, thus resulting in decreased potassium concentrations in the soil solution (Zhang et al., 2007). Hence, plants growing in acidic soils may exhibit symptoms of potassium deficiency such as stunted growth, leaf yellowing and diminished yield (Uchida, 2000). Yet, the effects of soil acidity on potassium availability can be complex and variable based on the soil types and plant species. In rare instances, the breakdown of specific minerals in the soil can release potassium into the soil solution, hence increasing the availability of potassium to plants. In addition, several plant species have evolved to be able to survive and even thrive in acidic soils and may have systems in place to promote potassium absorption and utilization under these conditions (Ghorbani et al., 2018; Padmavathiamma et al., 2008). Hence, it is essential to comprehend the specific requirements of the plant species being cultivated and to carefully manage soil acidity to guarantee that potassium levels are adequate for plant growth and health. Soil testing can assist in assessing soil pH and potassium levels, as well as advise on the use of suitable soil amendments to improve soil fertility and plant growth.

2.5 Soil Amendments Utilization in Acidic Soil

Soil amendments have been used in the tropics to improve soil chemical properties and nutrient bioavailability. There is a global interest to ameliorate acidic soils and one method to accelerate soil acidification is through liming. Calcium carbonate sourced from liming materials reacts with H^+ to produce OH ions, hence lowering the soil acidity levels (Ulrich, 1991). Liming has beneficial effects on soils such as enhanced nutrients and biota as well as improving plant productivity and quality (Holland et al., 2018). Besides liming,

the use of organic amendments has also gained great attention. The effect of organic soil amendments was observed in many studies. For instance, natural materials from animals or plants such as household waste, crop residue and livestock manure can act as compost and influence the physical, chemical and biological properties of the soil (Bouajila et al., 2011; Tejada et al., 2006). Numerous publications have also remarked on the potential benefits of biochar addition. Biochar has been shown to raise soil pH and other critical macro-elements, stimulating the soil to sequester carbon and reducing trace metals in leachate (Novak et al., 2009). The use of lime to neutralize acidity in high-input agriculture has resulted in a significant decrease in the area under cultivation and remarkable yield improvements in developed countries. Long-term flocculation of clay particles through compression of the double layers between clay particles is enhanced by liming agents, thus maintaining the soil pH (Haynes et al., 1998).

2.5.1 Lime

Raising the soil pH is the main objective of applying lime on agricultural land. Liming of acidic soils is a long-term worldwide practice of restoring soil acidity in agriculture (Juhrian et al., 2020). Applying lime to acidic soil in an adequate amount leads to a variety of biogeochemical changes that are beneficial for boosting crop yields on acidic soils. Neutralizing soil acidity, improving soil physical condition, solubility and leaching of heavy metals, effects on soil microbiome and biological process, improving soil nutrients profile, reducing phosphorus immobilization and increasing the supply of calcium and magnesium are among the effects of liming on agricultural crops (Mahmud et al., 2022).

A study by Opala (2017) and Dawid et al. (2017) shows that the application of lime can significantly reduce the exchangeable acidity, increased the soil nutrient availability, soil pH and plant yield. In addition, lime also can increase the fruit yield and has an

obvious effect in increasing leaf concentration of P (Oluwatoyinbo et al., 2005), which might be a result of lime's solubilizing effect on soil P and the consequent uptake of the latter by the plant (Naidu et al., 1990). By improving the soil health and quality during vegetation with lime, Mediterranean shrubs like *Retama sphaerocarpa* and *Myrtus communis* also recorded significant phytostabilization.

Lime applied at the recommended rate increases the soil pH, base saturation including Ca and Mg content and reduces Al concentration. According to Fageria et al. (2005), the acidity-neutralizing reaction of dolomitic lime [$CaMg(CO_3)_2$] occurs when Ca and Mg react with H on the exchange complex and H is replaced by Ca^{2+} and Mg^{2+} on the exchange site, thus forming HCO_3^-. HCO_3^- then reacts with H^+ to form CO_2 and H_2O to increase the pH.

Liming also causes biological changes in the soil including increased microbial biomass and enzyme activity (Haynes et al., 1988) while the crops grown in tropical acidic soil recorded an increase in the shoot dry weight, total root length and mycorrhizal colonization that improved P uptake (Nurlaeny et al., 1996). However, over-liming can create deficiencies in some of the micronutrients (Mortvedt, 2000) and may not be cost-effective (Mallarino et al., 2000). In addition, it can also lead to water pollution, nutrient imbalances, soil structure disruption, and ecological disturbances, all of which contribute to environmental pollution (Lu et al., 2015; Osemwota et al., 2000).

2.5.2 Food Waste Compost

Composting is regarded as an economical, simplified and cost-effective technique of managing solid organic waste that maximizes nutrient recycling. Local composting by households is regarded as a sustainable method, which is gaining great demand and has

become a major component in household waste in Southeast Asia, with 40-80% coming from food waste followed by paper, plastic, metal and glass (Curea, 2017). Municipal solid waste (MSW) in Malaysia is mainly disposed of at landfill areas whereby an anaerobic process releases traces of biogases such as CO_2, CH_4, H_2S and NH_3 that later contribute to the global warming process. Besides that, landfilling requires ample space and releases unpleasant odors and leachate that require further treatment. Chua et al. (2011), categorized the MSW from 2000 to 2010 based on the findings of several authors. The results showed that food and organic waste components ranged the highest every year from 32% to 68.4%. Thus, composting can provide a viable alternative method for managing food waste.

Composting food waste (FW) is one of the most effective methods for treating biodegradable waste components; it is also one of the potential waste management aspects for diverting trash generated from landfills while recycling organic materials through conversion into a valuable product. Moreover, FW compost has additionally been proven to increase the concentration of soil nutrients such as soil organic matter, dissolved organic carbon, total nitrogen, soil ammonium (NH_4^+) and nitrate (NO_3^-) (Dang et al., 2021).

Since 2009, University Malaya has initiated a project called Zero Waste Campaign (UM ZWC) in which one of its objectives is to develop a policy and an innovation system to divert solid waste from disposal in the landfill for resource and energy recovery. Through this project, 275 tonnes of food waste were able to be treated and composted (Yusoff, 2018). UM ZWC used the Takakura Composting Method (Figure 2.3) as an appropriate processing technology for the degradability of an organic portion (Carvajal-Millan et al., 2021).

Figure 2.2: UM ZWC Food waste compost used in this study

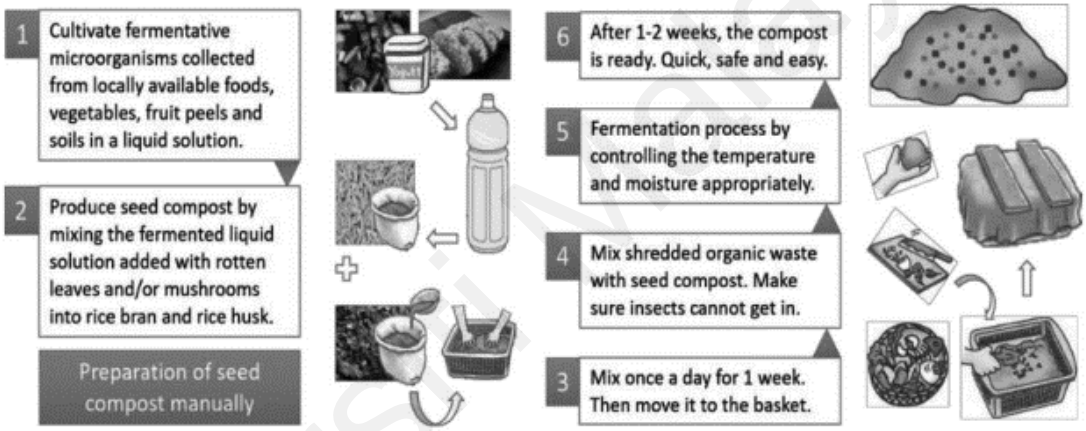


Figure 2.3: Takakura Composting Method Process (Nuzir et al., 2019)

Beesley (2010) reported that compost could improve vegetation, reduce compaction, protect against erosion and binding heavy metals, rapid mobilization and vertical transport of trace metals. In addition, the application of compost on acidic soil has been found to significantly increase the soil pH and Ca, Mg, K, Na and Si ions of in situ soil solution and decreased the amount of toxic ions (Al, Mn, and Fe). However, according to Van Fan et al. (2018), FW compost has some limitations such as low C/N, high moisture content and low porosity that depends on the quality of the initial materials in the compost (Van Fan et al., 2018).

Recently, co-composting or co-application of biochar and other organic amendments

has been identified as an excellent management practice by compensating for the limitation of each amendment. Furthermore, the addition of alkaline biochar to soils neutralizes soil acidity and may increase the cation exchange capacity (CEC) and base saturation depending on the intrinsic properties of the soil and the raw material of the biochar (Nahdiyyah S.H. et al., 2015).

2.5.3 Palm Kernel Shell Biochar

Biochar is recognized as a charred substance produced for use in several environmental applications including energy production, soil and water quality improvement and climate change mitigation (Ahmad et al., 2014). According to Kong et al. (2014), biochar incorporated into agricultural soil can help mitigate climate change through stable carbon storage and reduction of greenhouse gas emissions. Many studies have reported on the use of biochar as an amendment for crop production and improving the chemical properties in highly weathered tropical soils (Jien et al., 2013). However, only a few studies have investigated the effects of biochar on soil physical properties and soil erodibility. In addition, according to Manickam et al. (2015), limited studies have reported on the effect of the acquisition of biochar in Malaysian soils.

Oil palm industries play a significant role in Malaysian economic development since Malaysia is the second-largest producer of oil palm in the world after Indonesia. Hence, a massive amount of waste is created from this industry such as empty fruit bunches (EFB), mesocarp fruit fibers (MF) and palm kernel shells (PKS) (Abdullah et al., 2013). According to the Economic and Industry Development Division, Malaysian Palm Oil Board (MPOB), statistics showed 4,575,710 tonnes of palm kernel shells were produced from January to November 2019, whereas the palm kernel shells output in 2019 was high with about 15.6% in 2019 compared to 2018 (“The Star,” 2019). As such, due to the high

potential of palm waste that comes from PKS alone, PKS biochar can be a promising source of biomass resources.

Research has reported that biochar from oil palm waste has ameliorative properties including the ability to absorb soil pollutants and to decrease the acidity of soils (Liew et al., 2018). The addition of biochar to soil results in the release of alkaline substances, which have the potential to mitigate the process of acidification (Luo et al., 2018). Hence, due to such promising features, researchers have started to examine the use of palm kernel (PK) biochar (Figure 2.4) as a growth medium in ornamental plants (Abdullah et al., 2021).

Figure 2.4: Palm kernel biochar used in this study

2.6 Plant Cellular Antioxidant and Oxidative Stress Indicators in Plants

The presence of ROS in stressful environments results in the accumulation of ROS, which can trigger harmful oxidative processes such as lipid peroxidation, chlorophyll and betalain bleaching and protein oxidation. Plants have evolved enzymatic and nonenzymatic defensive mechanisms for scavenging and detoxifying ROS, resulting in an antioxidant defense capacity (Das et al., 2014). Stress environment stimulates the

active accumulation of solutes (such as proline, α-tocopherol and polyphenol) to protect them from oxidative damage and enables the plants to maintain a positive turgor pressure, which is required for stomata aperture and gas exchange (White et al., 2000). Besides that, non-enzymatic antioxidants such as leaf pigments, ascorbic acid, carotenoids, phenolics and flavonoids have a protective role in preventing ROS generation (Das et al., 2014).

Thus, there are several general types of response to abiotic stress. The typical effects are unbalanced water uptake (salinity), stomatal malfunctioning (temperature), diverse ionic exchange (waterlogging), stunted growth (drought and metals/metalloids toxicity) which lead to oxidative stress, damage in leaf and root ultrastructure, disruption of amino acid metabolisms, alteration of thiol biosynthesis and changes in osmotic metabolites and phloem inactivity during nutrient imbalance (Raza, 2021). Excessive accumulation of ROS (hydrogen peroxide, H_2O_2; superoxide, O_2^-; hydroxyl radical, OH^-; and singlet oxygen, $^1O_2$) and malondialdehyde are enhanced under abiotic and/or biotic stresses, which can cause oxidative damage to plant macromolecules and cell structures, leading to inhibition of plant growth and development, or even death. Among the various ROS, freely diffusible and relatively long-lived H_2O_2 acts as a central player in stress signal transduction pathways (Hossain et al., 2015). These pathways can then activate multiple acclamatory responses that reinforce resistance to various abiotic and biotic stressors. To utilize H_2O_2 as a signalling molecule, non-toxic levels must be rapidly altered by regulating the balance between H_2O_2 production and its scavenging rates (Zhang et al., 2016).

M. malabathricum L. is often described as an acid tolerant plant (Dorairaj et al., 2020). There are few reports related to the effect of environmental stress on secondary

metabolites of different plant species (Yadav et al., 2021). However, there is a lack of information available on *M. malabathricum* L., particularly with regards to chlorophyll, ROS markers (lipid peroxidation and H_2O_2), compatible solutes, non-enzymatic antioxidants (e.g proline), total carotenoid, reduced ascorbic acid, soluble protein, phenolics and flavonoids, as well as total antioxidant activity. Despite the growing interest on the effect of abiotic stress (acidic soil) on plant's ROS accumulation and its modulation through utilization of soil amendments, their specific effects on *M. malabathricum* L. remain largely unexplored.

2.7 Plant Phytohormone Secretion in Response to Stress Environment

Phytohormones play a critical role in regulating plant responses to abiotic stress, thus allowing the plants to survive under stressful conditions that result in reduced growth to allow the plants to focus their resources on surviving the stress (Skirycz et al., 2010). Abiotic stresses frequently resulted in changes in the production, distribution or signal transductions of growth and stress hormones, which may promote the activation of specific protective mechanisms (Eyidogan et al., 2012). The build-up of ROS during abiotic stress also influences the level and function of various plant hormones including abscisic acid (ABA) and auxin. For example, Du et al. (2013) showed that the endogenous levels of indole-3-acetic acid (IAA) were differentially regulated by abiotic stresses in rice. Besides, as investigated in another study, the salt-resistant maize significantly enhanced indole-3-butyric acid (IBA) concentrations in growing leaves in response to salinity stress conditions while maintaining IAA concentration in roots. Overall, this indicates that plant phytohormones play important roles in plant responses to environmental stresses.

2.7.1 Auxin

Auxin is important in plant growth, organ formation and plant responses to environmental stimuli. Indole-3-acetic acid ($C_{10}H_9NO_2$), or IAA, the predominant form of auxin in most plants, is synthesized from tryptophan or indole primarily in leaf primordial and young leaves including in developing seeds (Davies, 2010). Auxin can determine plant root development, which successively influences the plants' capacity to take up nutrients for water uptake and anchorage. It has also been recently reported that auxin biosynthesis genes play a role in root formation, essentially from embryogenesis (to initiate a root meristem) to form a functional root system consisting of a primary root, lateral root branches and adventitious roots. Moreover, auxin is also a key mediator for the adaptation of plants to environmental stressors, for example, in the regulation of root development and root system architecture (Kazan, 2013; Olatunji et al., 2017).

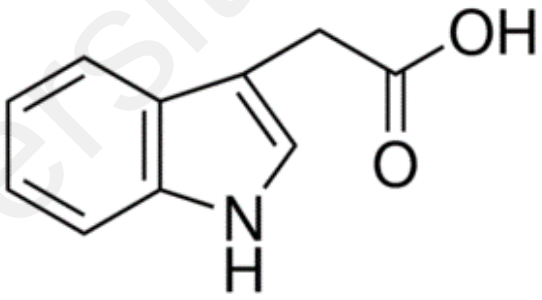


Figure 2.5: Chemical structure of Indole-3-acetic acid (IAA)

Furthermore, auxin is produced in plant tissues in unfavorable conditions. The interactions between ROS and auxin influence how plants respond to environmental challenges. As summarized by Tognetti et al. (2012), the crosstalk between ROS and auxin can be categorized by auxin biosynthesis, auxin metabolism and auxin redistribution and transport. A study by Kasahara (2016) also discovered that IAA levels are elevated in young tissues, which typically exhibit enhanced stress tolerance.

The low pH value is another example of an environmental stressor that can influence IAA biosynthesis (Ona et al., 2003). The pH, particularly exogenous pH, is among the highly variable environmental factors affecting the root which influences the capacity of plants for nutrient acquisition and further reduces root growth. However, the role of endogenous IAA distribution and transport in the leaves and roots of plants grown in acidic soil affected by amendments in the soil is particularly unclear and very few reports on its effects are available in the literature.

2.7.2 Abscisic Acid

Abscisic acid (ABA) plays an essential role in stress conditions. When plants are exposed to abiotic stress, phytohormone ABA acts as an endogenous messenger, regulating the plants' water status (Swamy et al., 1999). Through a combinational technique, ABA is expected to regulate stomatal closure within and outside guard cells. ABA functions are associated with not only stomatal closure but also water penetration and hydraulic regulation to allow for systemic adaptation to water stress (Fang et al., 2019; Majeed et al., 2011).

During the exposure of plants to stress conditions, ABA concentrations increase as a result of increased biosynthesis, the release of active ABA from conjugated forms or a decrease in degradation (Boursiac et al., 2013). Stress conditions such as drought and high salinity enhance the accumulation of ABA resulting in stomatal closure (Mittler et al., 2015) and altered gene expression (Shinozaki et al., 2007). Closure of the stomata further decreases transpiration and water loss resulting in lower gas exchange and photosynthetic activity. ABA and ROS may also form a positive amplification loop that regulates both stomatal function and gene expression in times of stress (Mittler et al., 2015).

2.8 Mass Spectrometry-based Metabolomic Studies on Plant Grown Under Stress

The term ‘metabolome’ was first coined in 1998 by Oliver et al. when he and his research team wrote a review article on genomic analysis on yeast. Genomics, as part of the omics disciplines, investigates the DNA sequence of organisms, while transcriptomics, proteomics and metabolomics provide valuable insights into the molecular composition and functions of living systems. Since metabolomics is at the end of the cellular regulatory processes, the changes can be seen as valuable information related to molecular or environmental stresses (Wolfender et al., 2013). There has been an enormous interest in investigating the link between abiotic factors such as temperature, salinity, water, radiation, chemical and mechanical stress with metabolites level (Akula et al., 2011). Environmental stress led to an increased production of free radicals and other oxidative species in plants (Xie et al., 2019). It also promotes the production of damaging active oxygen species within the cells. In addition, nutrient stress also has a marked effect on phenolic levels in plant tissues (Akula et al., 2011). Phenolic compounds is an essential component of plant defense that functions as an antioxidant to break down free radical chains that can cause lipid peroxidation. A number of genes are affected, which resulted in increased levels of several metabolites and proteins, in which some of them are responsible for conferring a certain degree of defense against these environmental stresses (Zhang et al., 2021). Integrating signaling molecules such as salicylic acid, jasmonic acid and their derivatives is frequently used to induce the expression of genes involved in secondary metabolism (Naikoo et al., 2019).

Plant metabolomics is a valuable tool for understanding the biochemical changes that occur in plants during stress. Nowadays, with the comprehensive recent technologies in analytical and multivariate data analysis, plant extracts can be analyzed for their

metabolite annotation and metabolite fingerprinting. Studies on metabolomics are crucial because they allow for the characterization of plants' physiological responses to various environmental stresses. A study by Nam et al. (2019) demonstrated that the induction of drought stress significantly altered the accumulation of primary and secondary metabolites in soybean using GC-MS and LC-MS/MS analyses. In another study, Wang et al. (2021) discovered a similar finding by demonstrating that the changes in metabolite of *Ricinus communis* cotyledons and roots are distinct under salt stress during the early seedling stage. A study by Hossain et al. (2017) was able to determine the role of photosynthetic organelle through their investigation on metabolite profiling of leaf tissues and chloroplast of *Beta vulgaris* L. (sugar beet) subjected to salinity via GC-MS analysis. Currently, very limited reports can be found on the metabolite profiles of plants grown under acidic soil condition with organic amendments. Thus, in order to fill in the gaps of our knowledge on the physiology underlying acidic tolerance, specific metabolite patterns can be linked to stress tolerance and may indicate mechanisms involved in the management of soil acidic stress.

LC-MS is suitable for detecting poor thermal stability and non-volatile metabolites due to its wide measurement ranges, efficient ion production and enhanced selectivity. As a result, it is regarded as one of the most applicable and versatile metabolomics methods (Rhoades et al., 2016). LCMS is a powerful analytical technique that has several advantages compared to other methods such as nuclear magnetic resonance (NMR). Automated software is needed to identify peaks from the raw data, align the peaks among different treatments and replicates to identify and quantify each metabolite (Ghatak et al., 2018). Classical chemometrics tools using Multivariate data analysis (MVDA) such as principal component analysis (PCA) and partial least square (PLS) are commonly used to group metabolites according to their similarities (Wolfender et al., 2013) as it generates

complex and large dataset that is difficult to analyze. Various open-source software is used as tools for analysis such as MetaboAnalyst, MZmine and XCMS and also pathway-related databases such as KEGG and SIRIUS. This can help to identify which molecules are altered in response to stress and how these changes vary between different types of stress. The use of multivariate as a data mining method has been demonstrated to be a powerful tool for metabolomics studies. The goal of metabolomics studies is to gain a better understanding of the biochemical changes that occur in plants during stress. This knowledge could be useful for future studies aimed at understanding the unknown metabolite responses during stress response in plants.

Upon reviewing the gathered information, certain conclusions can be drawn. Firstly, in the case of *M. malabathricum* L., there is an absence of evidence regarding the study on the physiological performance focusing on PK Biochar and FW compost as soil amendments. Additionally, the nutrient content, encompassing macro and micro nutrients in both amendments, has not been adequately elucidated. It is crucial to note that explaining growth focusing on anatomical features is insufficient. The growth process also involves biochemical changes, particularly in acidic soil conditions, such as the secretion of phytohormones and metabolic activities.

CHAPTER 3: METHODOLOGY

3.1 Methodology Outline

The outline of the methodology for this research project is shown in Figure 3.1. In order to examine the effects of soil amendments on the growth and physiological characteristics of *Melastoma malabathricum* L. on tropical acidic soil, a six-month study was conducted at the glasshouse located at Rimba Ilmu, Institute of Biological Sciences, Universiti Malaya, Kuala Lumpur, Malaysia (3° 7' 52.1076" N, 101° 39' 25.218" E). The maximum photosynthetically active radiation (PAR) received by the glasshouse was 2000 µE mol m^{-2} $s^{-}1$, the atmospheric temperature of the glasshouse varied from 25-28°C while the relative humidity (RH) recorded was 65-90%. The plants were also harvested to examine their growth performance, nutrient contents and physical characteristics. The cumulative ranking analysis was determined using growth performance, nutrient analysis and physiological performance to determine the best two treatments. In this assessment, each parameter was ranked from a maximum of 8 (best performance) to a minimum of 1 (lowest performance) using a weighted value. Finally, each value was added together and labeled as a cumulative ranking analysis value. The best two treatments, including control, were chosen for the next six months of the glasshouse study.

In the next phase of this study, the quantification of auxins in the aerial and underground organs (shoots, stems and roots) of *M. malabathricum* L. from the best two treatments (in comparison to control) was determined using dispersive liquid-liquid microextraction followed by HPLC. Additionally, the endogenous ABA was investigated while ROS signaling, secondary metabolite production and antioxidant capacity were also measured and compared.

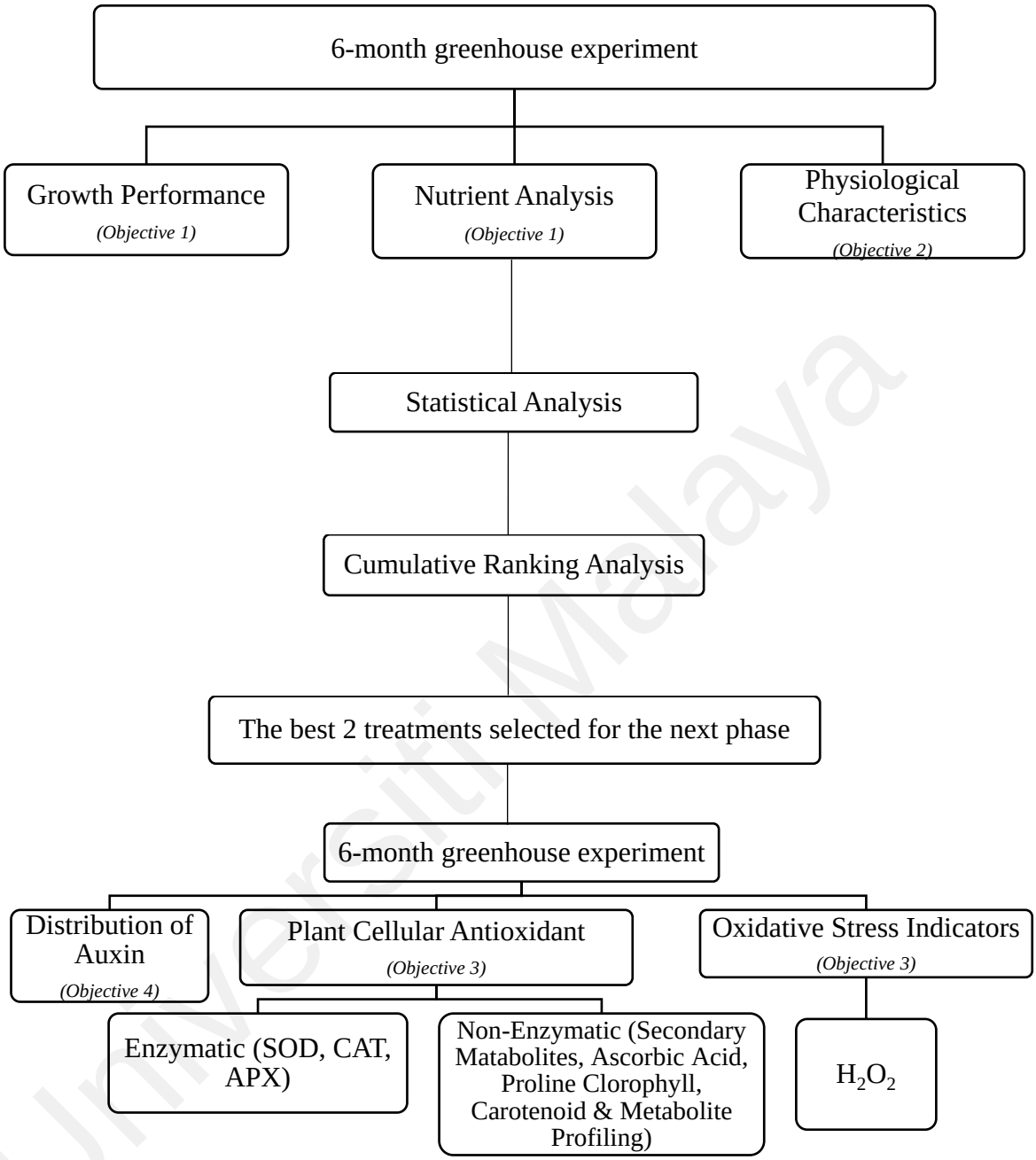


Figure 3.1: The methodology outline used in this study

3.2 Plant Materials and Experimental Design

M. malabathricum L. seedlings were obtained from a nursery in Sungai Buloh, Selangor, Malaysia. Initially, the average height of the seedlings was 10 ± 0.1 cm tall. The plants were grown in a polybag 26 cm x 20 cm filled with 8164 cm^3 soil and were arranged in a Complete Randomized Design (CRD) (Figure 3.2). The plants were watered daily during the experiment to keep them turgid and to prevent water stress. There were eight treatments in this experiment, and each treatment comprised of six replications. The treatments were as follows:

a. Control (Q) – no additions of amendment to the soil.
b. Liming (L) –10 t/ha of ground magnesium limestone.
c. Compost (C) – 10 t/ha of food waste compost.
d. Biochar (B) – 10 t/ha of palm kernel biochar.
e. Combination of liming and compost (CL) – 50% liming and 50% food waste compost.
f. Combination of liming and biochar (BL) – 50% liming and 50% palm kernel biochar.
g. Combination of compost and biochar (BC) – 50% food waste compost and 50% food waste compost.
h. Combination of liming, compost and biochar (BCL) – 33.3% palm kernel biochar, 33.3% food waste compost and 33.3% liming.

Figure 3.2: Experimental layout in the glasshouse following CRD.

3.3 Herbarium Specimen Preparation for Plant Identification

The plant's stem, flowers and fruits were carefully cut and placed inside a folded newspaper. To accommodate the entire plant material, the leaves were mounted dorsiventrally and in a "V" shape. The mounted specimens were sandwiched between cardboard frames and then heated at 40°C for two weeks. The dried specimen was then mounted using natural glue or stitched onto a 28 cm x 42 cm herbarium sheet. To ensure maximum contact with the glue, a few pieces of paper and appropriate weights were placed over the mounted specimen. The specimen was identified through comparison with the specimens in the Herbarium of the Forest Research Institute Malaysia (FRIM), Kepong, Selangor, which were deposited with the specimen voucher (PID 100720-09).

3.4 Effects of Different Soil Amendments Towards Growth and Nutrient Uptake of *M. malabathricum* L. in Tropical Acidic Soil Conditions.

3.4.1 Plant Height and Stem Diameter

Plant height and stem diameter were measured in three replicates per treatment using a flexible meter ruler and vernier caliper, respectively at the initial time of the experiment and at every three-month interval until harvest, which occurs after a six-months planting period.

3.4.2 Leaf Area Index

Leaf area index was determined at the initial, middle and final stages using a Ceptometer (AccuPAR LP-80 Decagon Devices, Inc, USA). The average reading per plant, above and below the plant was taken using a 1-meter long bar with 80 sensors divided into eight segments.

3.4.3 Root Profiles

Root measurement in different forms was carried out using a root scanner (WinRHIZO Pro v. 2008a, Regent Instruments Inc., Canada). The roots were washed thoroughly, air-dried and kept in a fridge. It was ensured that no water droplets existed on the roots before scanning was carried out. In addition to the root length, the volume and diameter of the root axes were also determined. The measurements were taken in triplicates and then used to calculate the mean values.

3.4.4 Plant Biomass

After 6 months of the experiment, the leaves, stems and roots of the plants were separated and oven-dried at 80°C for 48 h to obtain constant weights. The average measurements were calculated based on three replications.

3.4.5 Plant Nutrient Analysis

Leaf sampling was conducted after harvest and its nutrient content (N, P, K, Ca, Mg) was determined. Subsequently, total N was measured using the combustion method (Pansu et al., 2007) via Elemental Analyzer (Elementar, Vario Macro, Hanau, Germany). Other elements underwent dry ashing and digestion with the nitric acid method (Vogt et al., 2015) and were further determined spectrophotometrically using Inductive Coupled Plasma Optical Emission Spectrometer (Perkin Elmer Avio 200 ICP-OES, USA).

a) Preparation of Leaf Samples

The samples were collected, washed with distilled water and blot-dried before being dried completely in an oven (Memmert, Germany) set to 70°C. The finely ground dried leaf samples were then stored in an airtight container until further analysis.

b) Determination of Total N

The total N content of the leaf samples was determined by dry combustion using an Elemental Analyzer or EA (Elementar, Vario Macro, Hanau, Germany). The procedures were based on the guidelines in the equipment's manual (Elementar Analysensysteme GmbH, 2004). A total of 20 mg of finely ground leaf samples were weighed into tin foil cups and folded to seal before being put into a machine and the start button was pressed

to analyze the samples. Each sample was individually flushed with helium carrier gas to remove atmospheric nitrogen resulting in a zero-blank sampling process. Catalytic combustion occurs at a temperature of up to 1200°C. Oxygen is injected via a lance directly at the hottest spot, which results in the optimum combustion of the combustion gases.

c) Determination of Total Elements

Other trace elements (P, K, Ca, Mg) in the leaf samples were determined using the dry ashing method (Miller, 1998). A total of 1 g of finely ground samples were placed in a porcelain crucible and heated in a muffle furnace (Carbolite, Germany). The furnace temperature was slowly increased from room temperature to 600°C for 6 h and left to cool overnight. The grey ash residue was dissolved with a few drops of reverse osmosis water before 2 ml of hydrochloric acid (37% HCl) was added and heated on a hotplate (DLAB HP550-S, USA) at 120°C until dryness for 10 min. The residue was dissolved in 10 ml of 20% (v/v) HNO_3 and heated in a water bath (DFD-700, China) at 97 to 100°C. The solution was filtered through Whatman No. 42 filter paper and the residue was then washed with reverse osmosis water, filtrated and combined with the extract solution sample in a 100 ml volumetric flask. The filtration process was left overnight for the filter paper to completely dry and then diluted to 100 ml with reverse osmosis water. The filtrated solution was analyzed using Inductive Coupled Plasma Optical Emission Spectrometer (Perkin Elmer Avio 200 ICP-OES, USA). The same procedure was used to perform a blank digest.

d) Standard of Total Elements

Prior to analyzing the samples, the ICP-OES was calibrated using standard solutions. Each element's standard was prepared using a different chemical. Table 3.1 and the equation below show the equation and details of the chemicals used to prepare 1000 ppm standard solutions. All chemicals were purchased from Merck, Germany.

$$\text{Mass of chemical (mg)} = \frac{\text{molecular weight } \left(\frac{\text{g}}{\text{mol}}\right) \text{ x standard concentration}\left(\frac{\text{mg}}{\text{L}}\right) \text{ x volume of volumetric flask(L)}}{\text{atomic weight } \left(\frac{\text{g}}{\text{mol}}\right)} \quad (3.1)$$

Table 3.1: Chemicals and the mass of chemicals used

Element	Chemical	Molecular formula	Mass of chemical (g)
K	Potassium dihydrogen phosphate	KH_2PO_4	6.9614
Mg	Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$	20.2814
Ca	Calcium chloride dihydrate	$CaCl_2.2H_2O$	7.3363

3.4.6 Soil Analysis

After harvesting, the soil samples were air-dried in the glasshouse, sieved through a 2.00 mm sieve and keep in closed container for further analysis. The pH value of the soil was determined according to the method of 10 g of soil and 25 mL of distilled water (1:2.5 ratio). The total nitrogen content of the soil was determined using the Kjeldahl distillation method (Bremmer et al., 1982), the Bray and Kurtz method for determining the P element (Bray et al., 1945) and the leaching method (Allen et al., 1974) for determining K, Ca, Mg Na and CEC. The aqua regia method was used to determine Zn, Fe, Mn, Cu. Cd B and Al. The content of the macro and micro nutrients (P, K, Ca, Mg, Na, Zn, Fe, Mn, Cu,

Cd, B, Al) was determined using the Inductive Couple Plasma Optical Emission Spectrometer (ICP-OES).

a) Preparation of Soil Samples

At three different points for each soil treatment, soil samples were randomly collected at 15 cm of soil depth. Subsequently, the air-dried soil samples were crushed with a mortar and pestle and sieved using a 2.0 mm laboratory siever (Endecotts, England) to determine the soil pH. The remaining samples (2.0 mm) were then sieved through a 0.25 mm laboratory siever (Endecotts, England) and stored in an airtight container for further analysis, which included soil pH measurement, determination of particle size, the determination of Total N, determination of available P, determination of macronutrients in soil, determination of micronutrients in soil, and determination of Cation Exchange Capacity (CEC).

b) Soil pH Measurement

The soil pH was determined in a supernatant suspension of a 1:2.5 mixture of soil and distilled water (Shariff et al., 1992). A total of 10 g of air-dried, ground and sieved soil was weighed into a plastic weighing boat using an electronic balance. The mixture was added with 25 ml of distilled water and an orbital shaker was used to shake the mixture for one h at 2.5 x 100 rpm (ZP-200, Meditry Instrument, China). The pH meter (PB-10, Sartorius, Germany) was calibrated prior to taking pH readings with buffer solutions of pH 4, pH 7 and pH 10. Triplicate pH values taken after harvest were then recorded and averaged.

c) Determination of Particle Size

The particle size determination (PSD) was performed using a laser analyzer Mastersizer 2000 with a Hydro MU adapter within a measurement range of 0.02 – 2000 µm. Firstly, the soil samples were oven-dried at 105°C. The ground-dried soil samples were then sieved through a 2 mm laboratory siever (Endecotts, England). An 800 cm^3 beaker containing 650 ml of distilled water was used in the Hydro MU unit. Two light sources with wavelengths of 633 nm and 460 nm were employed by the Mastersizer device. The determination of the quantity of soil sample added into the measuring system relies on the parameter known as "obscuration', which is measured by the apparatus during the addition of each sample, representing the extent to which the particles being measured with a range of 10% and 20% as recommended by the manufacturer (Ryżak et al., 2011)

d) The Determination of Total N

The Kjeldahl method was used to determine the total nitrogen content of the soil (Bremmer et al., 1982). In a digestion tube, 0.30 g of soil (0.25 mm) and 1 Kjeldahl tablet was added. Subsequently, 3 ml of acid (salicylic and sulfuric acid) was added to the same digestion tube and heated in a digestion block for one h at 260°C and further increased to 360°C for the remaining 2 h. A total of 10 ml of distilled water was then added and mixed using a vortex (Corning, USA) after being left overnight. After filling the samples to 50 ml with distilled water, they were sealed with parafilm, shaken and then filtered using Whatman No. 42 filter paper before being collected in a plastic vial. A total of 5 ml of samples were then transferred to a Buchi flask, distilled with a Buchi distillation unit K-355 (BUCHI, Switzerland) and the distillate was collected in a conical flask containing 10 ml of 3% (v/v) boric acid and four to five drops of indicator (0.10 g of methyl red and

0.05 g of methylene blue in 100 ml of ethyl alcohol). Titration (Mettler Toledo Titra Mate10, Switzerland) with 0.1 N hydrochloric acid was performed until the solution changed colour from green to pink.

e) Determination of Available P

The element P content of the soil samples was determined using the spectrophotometric molybdenum blue method by Bray et al. (1945). In a 50 ml plastic vial, 2 g of dried-sieved soil samples were added. The plastic vial was then filled with 20 ml of 0.01 N NH_4Fl_2 and 0.03 N HCl. Subsequently, the samples were shaken for 30 min at a rate of 20 rpm using an orbital shaker (Lab Line, USA). A total of 5 ml of filtrate solution was then pipetted into a 50 ml volumetric flask, which was filled with 8 ml of reagent C. Finally, the samples were analyzed using UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, USA).

f) Determination of Macronutrients in Soil

The K, Ca, Mg and Na elements were determined using the ammonium acetate leaching method (Allen et al., 1974). Columns for leaching were prepared by inserting filter papers into the columns. In the leaching columns, 10 g of dried soil was packed. Subsequently, 100 ml of ammonium acetate (pH7) was added to the columns. The leaching columns were run continuously at a rate of one drop per ten seconds into a 100 ml volumetric flask until the columns were completely drained. This process typically took between 5 to 6 h. Afterward, ammonium acetate was used to mark the volumetric flask and the solution was filtered through the filter papers into a 100 ml plastic vial. The filtered solution was then transferred into a test tube. The ICP-OES (Perkin Elmer Avio 200 ICP-OES, USA) was used to analyze the samples.

g) Determination of Micronutrients in Soil

Trace elements of Zn, Fe, Mn, Cu, Cd, B and Al were determined using ICP-OES. A total of 10 g of dried-sieved soil samples were added to a 100 ml plastic vial, which was added with a 50 ml acid mixture (HCl and H_2SO_4). For 30 min, the samples were shaken at a rate of 2 rpm using an orbital shaker (Lab Line, USA). Finally, the filtered samples were analyzed using ICP-OES (Perkin Elmer Avio 200 ICP-OES, USA).

h) Determination of Cation Exchange Capacity (CEC)

Soil samples in the leaching columns were used to determine the total elements. The soil samples were washed with methyl spirit to remove any remaining ammonium and the methyl spirit was poured into a waste bottle and discarded. In the leaching columns, 100 ml of potassium sulfate was added and leaching was carried out at a rate of 1 drop per ten seconds into a 100 ml volumetric flask until the columns were completely empty. This process took approximately 5 to 6 h. Subsequently, the volumetric flask was marked with potassium sulfate. A total of 50 ml of the solution was transferred to a vessel tube for Buchi distillation unit K-355 (BUCHI, Switzerland) and three drops of the indicator were added to a conical flask containing 25 ml of boric acid. Before the program began, 1 ml of distilled water and 25 ml of NaOH were added to the conical flask. Following the completion of the distillation process, the solution was titrated (Mettler Toledo Titra Mate10, Switzerland) with 1.0 N of HCL until the light color changed to purple.

i) Standard of Total Elements

The ICP-OES was calibrated using the standard solution prior to analyzing the samples. The standard for each element was prepared using a different chemical. The

equation and details of the chemicals used to prepare 1000 ppm standard solutions are shown in Figure 3.2 and Table 3.2. All of the chemicals were supplied by Merck, Germany.

Table 3.2: The mass of chemicals used

Element	Chemical	Molecular formula	Mass of chemical (g)
K	Potassium dihydrogen phosphate	KH_2PO_4	6.9614
Mg	Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$	20.2814
Ca	Calcium chloride dihydrate	$CaCl_2.2H_2O$	7.3363
B	Boric acid	H_3BO_3	0.5730
Na	Sodium chloride	NaCl	2.5420

3.5 The Effects of Different Soil Amendments on Physiological Characteristics of *M. malabathricum* L.

3.5.1 Relative Chlorophyll Content

Every two months, the relative chlorophyll content was determined using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) at three distinct locations on one leaf per plant and the average value from three replications of reading was calculated (Liu et al., 2012). Additionally, SPAD measurements were taken between 09:30 h and 11.30 h, which is the peak of the photosynthesis process (Aimee et al., 2014).

3.5.2 Rate of Photosynthesis, Stomatal Conductance, Transpiration Rate and Water Use Efficiency

Between 09:30 h and 11:30 h, when the peak of photosynthesis occurred (Aimee et al.,

2014), a portable photosynthesis system (LiCor-6400; LiCor Inc., USA) was used to determine the rate of photosynthesis, stomatal conductance, transpiration rate and water use efficiency of the plants through fully expanded and mature leaves. For each of the six biological replicates, three technical replications were logged and recorded automatically. The initial and final readings were also recorded.

3.6 Cumulative Ranking Analysis

The cumulative ranking analysis was performed following Ganepola et al. (2021) with a minor modification on *M. malabathricum* L. plants grown under various treatments based on their development, nutrient uptake and physiological parameters. Each parameter was ranked using a weighted value ranging from 1 to 8. A maximum value of 8 was given to the best soil treatment parameter with the best performance whilst a value of 1 was given to the parameter with the lowest performance. Finally, each individual value was added together and marked as the ultimate cumulative ranking of each soil treatment with the species with the greatest value was chosen as the best. This screening process would assist in selecting the best two treatments that show comparatively better plant performance.

3.7 Effects of Different Soil Amendments Approaches on Plant Cellular Antioxidants and Oxidative Indicators

3.7.1 Analysis of Oxidative Stress Indicators

3.7.1.1 Determination of Hydrogen Peroxide (H_2O_2) Level

The standard protocol by Velikova et al. (2000) was used to determine the H_2O_2

content. Briefly, 0.07 g of leaf tissues were homogenized in 5 ml of 0.1 % (w/v) trichloroacetic acid (TCA) in an ice bath. A total of 0.5 ml of supernatant was added to 0.5 ml of 10 mm potassium phosphate buffer (pH 7.0) and 1 ml of KI after centrifugation at 12,000 g for 15 min. At 390 nm, the absorbance of the supernatant was determined. The content of H_2O_2 was then determined by comparing it to a previously created standard calibration curve using a series of H_2O_2 concentrations.

3.7.2 Measurement of Antioxidant Enzyme Activities

The plant cellular antioxidant machinery has two types, which are enzymatic components and non-enzymatic antioxidants. The enzymes are localized in the different subcellular compartments and are comprised of the antioxidant machinery including Superoxide Dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX). Tissue extraction of the samples was prepared following Bradford (1976). The protocol of Giannopolitis et al. (1977) was followed to determine SOD while CAT and APX enzymes were determined following the protocol of Chance et al. (1955) and Nakano et al. (1981), respectively.

3.7.2.1 Superoxide Dismutase (SOD)

The superoxide dismutase (SOD) enzyme activity was measured using the leaf, stem and root samples. First, the samples were subjected to an extraction procedure using a cold extraction buffer consisting of 100 mM of potassium phosphate pH 7.0 and 0.1 mM of EDTA (Giannopolitis et al., 1977). A total of 50 µl of supernatant was mixed with 50 mM of phosphate buffer (pH 7.8), 13 mM of methionine, 0.1 M of EDTA, 75 M of nitroblue tetrazolium and 2 M of riboflavin to activate the reaction. The reaction mixture was illuminated with 20-watt fluorescent tubes and its absorbance at 560 nm was

measured.

3.7.2.2 Catalase (CAT)

The catalase (CAT) activity was measured following a method by Shimizu et al. (1984). To get the crude extract, the samples were pulverized in a 100 mM sodium phosphate extraction buffer (pH 6.8) and centrifuged. The process was initiated by the addition of 100 mM potassium phosphate buffer, 0.1 M EDTA and 20 mM hydrogen peroxide to the crude enzyme extract. A reduction in the optical density at 240 nm suggests a reduction in hydrogen peroxide and indicates the activity of the CAT enzyme. The enzyme activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1}.

3.7.2.3 Ascorbate Peroxidase (APX)

The ground samples were extracted in a 50 mM phosphate buffer (pH 7.8) in order to assess the enzyme activity of ascorbate peroxidase (APX) (Nakano et al., 1981). The reaction was initiated by mixing the crude extract with a reaction mixture consisting of 50 mM phosphate buffer, 0.2 mM EDTA, 0.5 mM ascorbic acid and 0.25 mM hydrogen peroxide. The absorbance was recorded at the start and end of the reaction at 290 nm. The molar extinction coefficient used for the calculation was 2.8 mM^{-1} cm^{-1}.

3.7.3 Measurement of Non-Enzymatic Antioxidant

The amount of various non-enzymatic antioxidants such as chlorophylls (a and b), carotenoids, proline and ascorbic acid, as well as the secondary metabolites including the total phenolic content (TPC), and total flavonoid content (TFC) were quantified. The distribution of the individual flavonoids as well as other secondary metabolites in the

aerial and underground plant organs were also evaluated using HPLC and LCMS, respectively.

3.7.3.1 Preparation of Samples and Extraction of Metabolites

The harvested plants were freeze-dried at -50°C in a Labconco freeze dryer (Labconco Corporation, MO 64132, United States). A total of approximately 2.0 g of freeze-dried samples were ground in liquid nitrogen using a chilled mortar and pestle. All extraction procedures were conducted in complete darkness. After homogenizing the samples, the samples were subsequently soaked in 60 ml of absolute methanol and incubated at 4°C for 48 h. Filtration of the extracts was carried out using Whatman No. 2 filter paper and the filtrate was then collected and stored at -20°C. Re-extraction and filtration of the residue were also performed. The extracts were combined and centrifuged for 5 min at 9000 rpm and 4°C (Universal 32 R centrifuge Hettich Zentrifugen, D-78532 Germany). Meanwhile, a portion of these supernatants was immediately used for subsequent pigment analysis to determine the total chlorophyll, carotenoid and anthocyanin contents (Ashokhan et al., 2019) while the remaining supernatants were concentrated to dryness using a Rotavapor® (R-3, Büchi Labortechnik AG, Switzerland) at 45°C. The solvent-free extracts were further adjusted to a concentration of 10 mg/ml with absolute methanol and stored at -20°C in an airtight glass vial until further analysis.

3.7.3.2 Measurement of Chlorophyll and Carotenoid Contents

For measurement of chlorophylls (a and b) and carotenoid contents, the absorbance of the sample extracts was spectrophotometrically measured at 665.2, 652.4 and 470 nm, in triplicate. Subsequently, the contents were calculated based on the formula by Lichtenthaler et al. (2001) as shown below.

$$C_a(\mu g/ml) = 16.72\ A_{665.2} - 9.16\ A_{652.4} \quad (3.2)$$

$$C_b(\mu g/ml) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$C_{(x+c)}(\mu g/ml) = \frac{(1000\ A_{470} - 1.63\ C_a - 104.96\ C_b)}{221}$$

3.7.3.3 Proline Content Assay

Proline content was determined using Bates et al. (1973) method by grinding 0.25g of leaf samples in 5 ml of sulfosalicylic acid. A total of 2 ml of glacial acetic acid and 2.0 ml of acidic ninhydrin were added to every 2.0 ml of plant extract. The mixture was shaken vigorously and placed in a water bath at 75°C for 60 min, and the reaction was later terminated in an ice bath. The proline contents were obtained from the chromophore layer by adding 4 mL of toluene to the reaction mixture. Additionally, the tubes were incubated for 20 min at room temperature in the dark to allow for the separation of the toluene and aqueous phase, and finally, the absorbance was read at 520 nm using a Multiskan GO plate reader (Thermo Scientific, Waltham, MA, USA).

3.7.3.4 Ascorbic Acid

The ascorbic acid was measured following the method by Cesar et al. (2018). A total of 10 mL of distilled water were used to extract about 600 mg of dried leaf powder. At 3000x g and 4°C, the homogenates were centrifuged for 15 min. A total of 2 g of 2,4-dinitrophenylhydrazine (DNPH), 230 mg of thiourea and 270 mg of $CuSO_4$ were dissolved in 100 mL of 5 M H_2SO_4 to prepare for DNPH reagent. Then, 75µL of DNPH reagent was added to 100 µL of 13.3% trichloroacetic acid, 25µL of deionized water and 200µL of sample extract. Blanks were prepared without the DNPH reagent. Samples were

incubated at 37°C in a water bath for 60 min. After incubation and the addition of DNPH reagent to the blanks, 500 µL of 65% H_2SO_4 was added to all reaction mixtures. The absorbance was recorded at 520 nm. The concentration of ascorbic acid was obtained from a standard curve with known concentrations of ascorbic acid (2.5–20 µg mL^{-1}). The ascorbic acid content was expressed in µmole per min per mg of dry weight.

3.7.4 Analysis of Secondary Metabolites Content

3.7.4.1 Phytochemical Screening

Chemical analysis of the methanolic extracts was carried out to determine the presence of bioactive secondary metabolites using the standard assay procedure described by Solihah et al. (2012) with minor modifications.

a) Test for Phenol

A total of 2 ml of methanol extract was placed in a 45-50°C water bath (WNB-10, Memmert, Germany). To the extract solution, 2 ml of 3% $FeCl_3$ was added, and the presence of phenols was indicated by the formation of a green or blue color.

b) Test for Tannins

A total of 1 ml of methanol extract was added to 1 ml of $FeCl_3$ solution at a concentration of 3% and the presence of tannins was indicated by a greenish-black precipitate.

c) Test for Flavonoid (I)

A total of 1 ml of methanol extract was gently shaken with 1 ml of 10% $(CH_3COO_2)^2Pb$ and the presence of flavonoids was indicated by the formation of a muddy brownish precipitate.

d) Test for Flavonoid (II)

A total of 1 ml of methanol extract was added to 10 ml of $FeCl_3$. The mixture was shaken, and the presence of flavonoids was indicated by the formation of a woolly brownish precipitate.

e) Test for Phlobatannins

A total of 1 ml of methanol extract was boiled in 2 ml of 1% hydrochloric acid and the presence of phlobatannins was indicated by the formation of a red precipitate.

3.7.4.2 Total Anthocyanin Content (TAC)

The pH differential method following Giusti et al. (2001) with some modifications was applied for the total anthocyanin content (TAC) assay. The sample extracts were separately diluted with two types of buffer: potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5 using the ratio of 1:4 (one part test portion and four parts buffer). The absorbance was read in triplicate at 520 and 700 nm using a Multiskan GO plate reader (Thermo Scientific, USA).

$$\text{Anthocyanin pigment content (mg/L)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \quad (3.3)$$

Where $A=(Abs_{510}-Abs_{700})pH\ 1-(Abs_{510}-Abs_{700})pH\ 4.5$
MW(cyanidin-3-glucoside)=449.2 g/mol
DF=dilution factor
ε=26,900

3.7.4.3 Total Phenolic Content (TPC)

The total phenolic content (TPC) of the methanolic extracts was determined using the method described by Singleton et al. (1999) with minor modifications. Briefly, 10µL of 10mg/ml methanolic sample extract was added with 75µL of diluted Folin-Ciocalteu reagent (FCR) and incubated at room temperature for 10 min. Prior to incubation with the combination, FCR was diluted with deionized water. The mixture was added with 75µL of 2% Na_2CO_3 and incubated for 45 min in the dark. The absorbance of the samples was read at 765 nm using a Multiskan GO plate reader (Thermo Scientific, Waltham, MA, USA). Gallic acid at six different concentrations (from 0.1 to 0.55 mg/ml, $r^2 = 0.99$) was used to prepare the standard curve. The TPC of the samples was expressed as mg of gallic acid equivalents/g dry weight (g GAE/g DW) of the extracts.

3.7.4.4 Total Flavonoid Content (TFC)

The aluminium chloride colorimetric method (Yusof et al., 2018) was used with slight modifications to determine the total flavonoid content (TFC) of the extracts. A total of 20 µL of 10 mg/ml extract samples were mixed with 60 µL of 70% methanol, 4 µL of 10% aluminium chloride hexahydrate, 4 µL of 1 M sodium acetate and 112 µL of distilled water. After 40 min of incubation, the absorbance of the mixture was measured in triplicate at 415 nm using a Multiskan GO plate reader (Thermo Scientific, Waltham,

MA, USA). A quercetin standard curve of concentration ranging from 0.15 to 0.40 mg/mL was plotted to calculate the TFC of the samples and the results were expressed as quercetin equivalents in g per gram of dry weight (g QE/g DW) of the extracts.

3.7.4.5 Determination of Individual Flavonoids Using HPLC

Isocratic reverse-phase HPLC was conducted using HPLC Agilent Technologies 1100 series model HPLC system (Waters, Milford, MA, USA) equipped with an autosampler, binary pump, injector, micro vacuum degasser, thermostat column compartment and a UV-Vis diode array detection (DAD) as previously described by Seo et al. (2016) with some modifications.

a) Preparation of Samples and Extraction

A total of 2.0 g freeze-dried (Labconco Corporation, MO 64132, United States) samples were ground into powder using a chilled mortar and pestle in liquid nitrogen. The extraction process used for the individual flavonoids was conducted as previously described by Seo et al. (2016) with minor modifications. A total of 2 g of each freeze-dried plant sample was extracted with 20 ml of 80% ethanol at room temperature and incubated for 72 h before filtering the sample. To concentrate the extracts, ethanol was evaporated, freeze-dried, and kept at -80°C. The crude extracts were then redissolved in ethanol, adjusted to a concentration of 10 mg/mL and filtered using a 0.2 μM membrane for further analysis.

b) Chromatographic Condition

The quantification of individual flavonoid content was conducted using HPLC, Agilent Technologies 1100 series model HPLC system (Waters, Milford, MA, USA). A ZORBAX SB-C18 column (5 µm, 4.6 x 250 mm, Agilent Technologist, USA) maintained at 30°C was used as the separation channel. The mobile phase was composed of 100% acetonitrile at a flow rate of 0.8 ml/min. Subsequently, the extracts were screened for six types of flavonoids namely flavone, kaempherol, myricetin, rutin and quercetin.

3.8 Metabolite Profiling of Leaf and Root Extracts of *M. malabathricum* L.

a) Sample Extraction

The samples were extracted following Nam et al. (2019) method. Briefly, the samples were freeze-dried and then ground to form a powder. A total of 100 mg of freeze-dried leaf tissue was added to 1 ml of methanol. The mixture was extracted in an ultra-sonic water bath (Elma Ultrasonic Cleaner P180H, Germany) for 15 min and then centrifuged for 10 min at 20000 g at room temperature. The supernatant was then passed through a syringe filter with a pore size of 0.2 µm and then transferred into a glass vial.

b) Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis and Data Processing

Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF MS system with dual ESI source was used to conduct LC-MS analyses in this study. The separations were performed using Agilent Eclipse XDB-C18 Narrow-bore column maintained at 25°C. The mobile phases consisted of 0.1% formic acid in water (solvent

A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.5 mL min^{-1}. Elution was programmed as a linear gradient increasing from 5 to 100% B in 25 min, stopped at 100% B for 5 min. The injection volume was 1 µL. The electrospray ionization (ESI) conditions were set with an ESI spray voltage of 4000 V. The drying gas was set at 300°C at a flow rate of 10L min^{-1}. A reference solution was used with the two ions having m/z of 121.0508 and 92266.0097 for mass calibration to eradicate systematic errors. The samples were screened using positive ionization modes. The results were then collected and analyzed with Agilent MassHunter Qualitative Analysis B.07.00 software. Metabolites were then detected through comparison with the databases and standard libraries including Metabolite Mass Spectral Database (METLIN), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Chemical Compound Database (CAS SciFinder).

3.9 Effects of Different Soil Amendment Approaches on The Distribution of Auxin

Auxin distribution in the samples was determined using high-performance liquid chromatography (HPLC) following Mwange et al. (2003) with minor modifications.

3.9.1 Chemicals and Reagents

Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Indole-3-propionic acid (IPA) and 1-Naphthaleneacetic acid (NAA) standards were purchased from Sigma-Aldrich while HPLC grade acetic acid ($C_2H_4O_2$), methanol (CH_3OH), acetone $(CH3)_2CO$, polyvinylpyrrolidone (PVP) and acetyl acetate ($CH_3COOC_2H_5$) were from Sigma-Aldrich. Milli-Q water with a conductivity of 18.2 MΩcm^2 was obtained via the Milli-Q Millipore filter system (Millipore Co., Bedford, MA, USA).

3.9.2 Preparation of Samples

The fresh samples were finely ground with liquid nitrogen. The samples were extracted at 4°C for 24 h with 5 ml of 65% acetone and polyvinylpyrrolidone (PVP) as an antioxidant. The supernatant was collected after 15 min centrifugation at 4000 rpm. The pellet was reextracted with 3 ml of 65% acetone and recentrifuged. The pooled supernatants were evaporated under a nitrogen stream at 45°C and the pH was adjusted to 3. The phytohormone purification was performed with 3 ml of pure acetyl acetate and passed through a C18 column. The dried precipitate of acetyl acetate after evaporating under a nitrogen stream was collected in 0.8 ml of 50% methanol. It was then subjected to HPLC analysis after filtration with 45 µl of a membrane filter.

3.9.3 Chromatographic Condition

Chromatographic separation was carried out on the Shimadzu Prominence UFLC system equipped with an autosampler, binary pump, degasser, column oven and UV/vis detector. Following filtration through a 0.45 mm membrane filter, the mobile phase was comprised of 50% methanol in double-distilled water with an addition of 1% acetic acid. The separation channel was maintained at 40°C with CNW Athena C18 column (2.1mm x 150mm x 3µm) while the UV wavelength detector was set to 280 nm. Each analysis required a 10 µL injection volume at a flow rate of 0.3 mL min^{-1}.

3.10 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistic 25 software (IBM Corporation, Armonk, NY, USA). A t-test was carried out between the initial and final planting treatment to analyze the significant differences at p-value ≤ 0.05. A one-way ANOVA was carried out to evaluate the significant differences among the means of

treatments at p-value ≤ 0.05. All one-way ANOVA treatment means were assessed by Tukey's post-hoc test. Pearson's correlation was performed to analyze relationships between the studied parameters. A biplot figure was generated using PAST software to examine the principal component analysis (PCA).

CHAPTER 4: RESULTS

4.1 The Effect of Soil Amendments on the Growth and Nutrient Uptake of *M. malabathricum* L. in Tropical Acidic Soil Conditions

4.1.1 Plant Height and Stem Diameter

The height and stem diameter of *M. malabathricum* L. in all treatments showed an increasing trend (Figure 4.1 and Figure 4.2) from initial to final planting phase. After six months, the mean height of *M. malabathricum* L. in B, BL, BC, BCL and C (31.18, 21.67, 21.60, 21.27 and 20.90 cm, respectively) were significantly greater compared to L, BCL and Q. The height of *M. malabathricum* L. grown in the soil treated with B achieved the highest increment (159.83%) whilst Q exhibited the lowest increment at 12.63%. All treatments also recorded an increment in stem diameter after six months with the greatest exhibited in B with a 30.88% increment compared to control followed by C and BC with 20.88% and 14.66%, respectively. BCL treatment recorded the lowest stem diameter increment with only 0.18%.

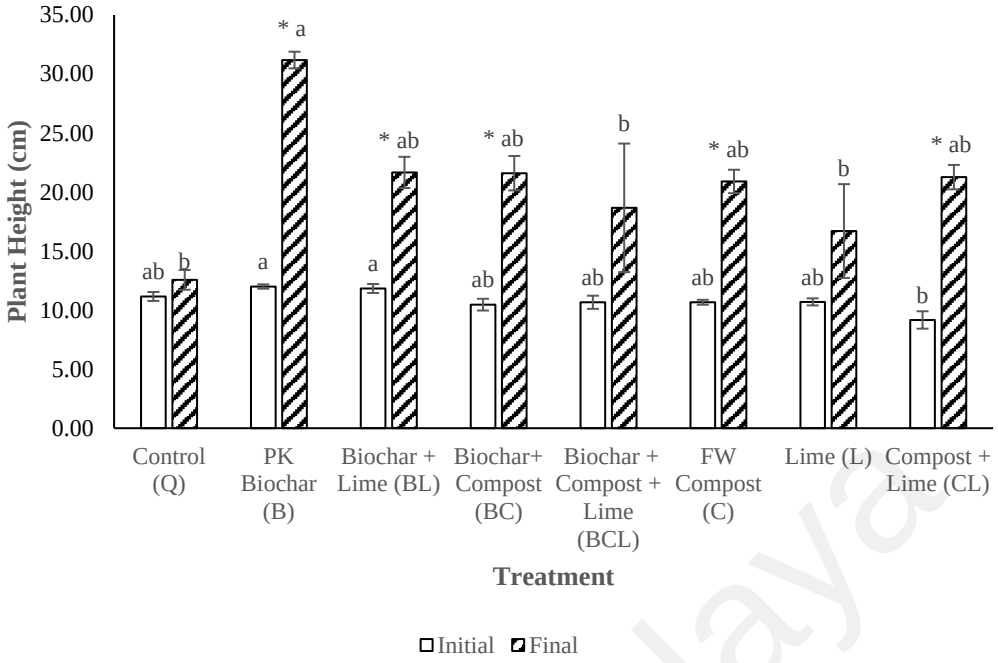


Figure 4.1: Plant height in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatment means during final planting at $p \leq 0.05$. The asterisk (*) denotes a significant difference between initial and final planting at $p \leq 0.05$.

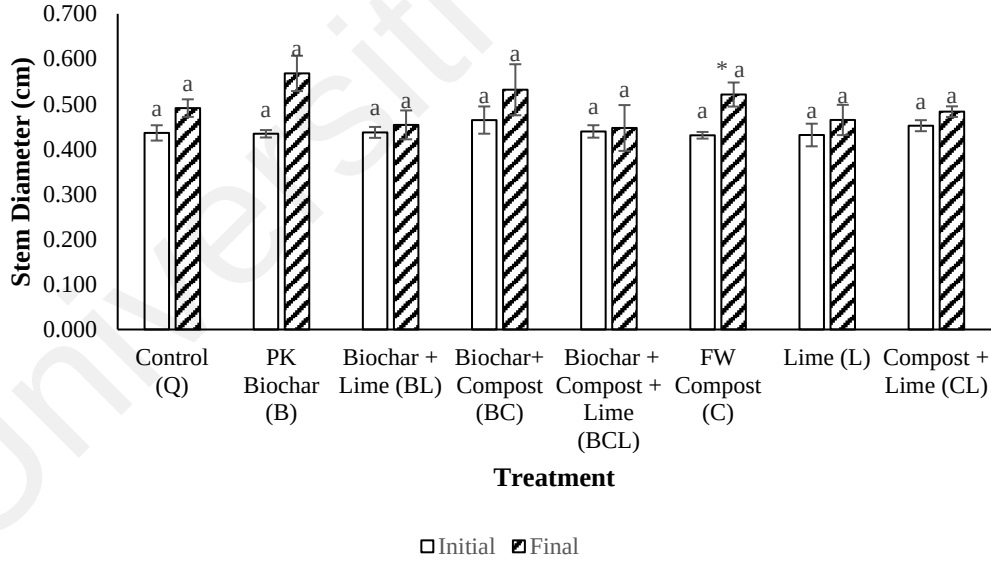


Figure 4.2: Stem diameter in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatment means during final planting at $p \leq 0.05$. The asterisk (*) denotes a significant difference between initial and final planting at $p \leq 0.05$.

4.1.2 Leaf Area Index

The leaf area index (LAI) of *M. malabathricum* L. increased with time throughout this study. An increasing trend was observed between the initial and final planting periods. The LAI of *M. malabathricum* L. grown in all amendments were comparable to that of control plants with no significant difference in the LAI during the final planting period in all treatments. During the final planting period, the LAI of *M. malabathricum* L. grown in the soil treated by B recorded the highest LAI whilst Q recorded the lowest LAI (Figure 4.3).

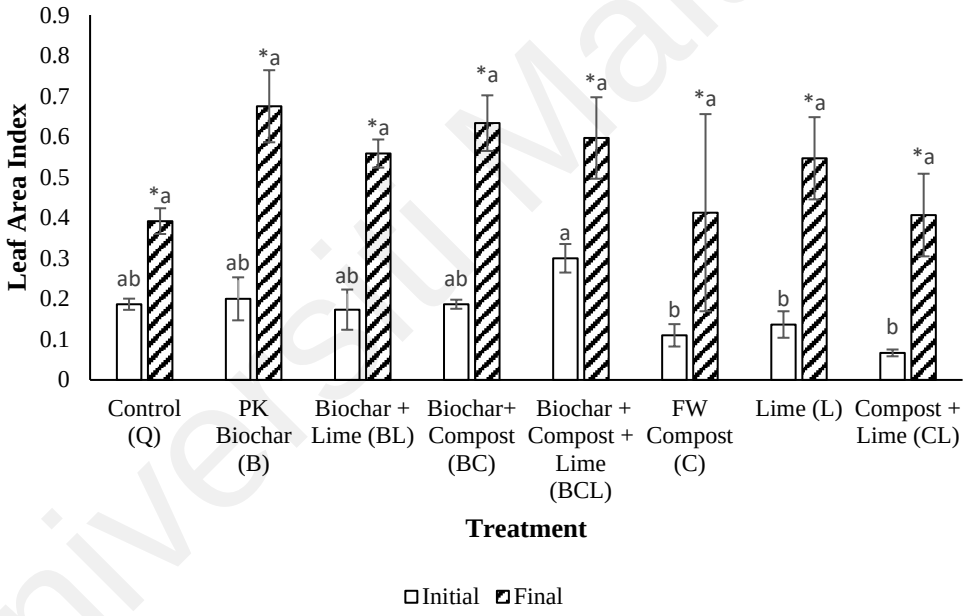


Figure 4.3: Leaf area index in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatments during final planting at $p \leq 0.05$. The asterisk (*) denotes a significant difference between initial and final planting at $p \leq 0.05$.

4.1.3 Root Profiles

Overall, the root length and root length density displayed a similar trend of response toward the treatments. Meanwhile, the root average diameter and root volume also

exhibited a similar trend of treatment response. *M. malabathricum* L. grown with B treatment displayed the highest root length and root length density which was 20.25% higher than those in the control, followed by BL and L treatments (Figure. 4.4a, b). However, data analysis revealed that the root average diameter and the root volume were significantly highest in the C treatment with 121.30% higher compared to the lowest treatment of BCL (Figure. 4.4c, d). The three combined amendments (BCL) had the lowest root average diameter and root volume after six months of observation.

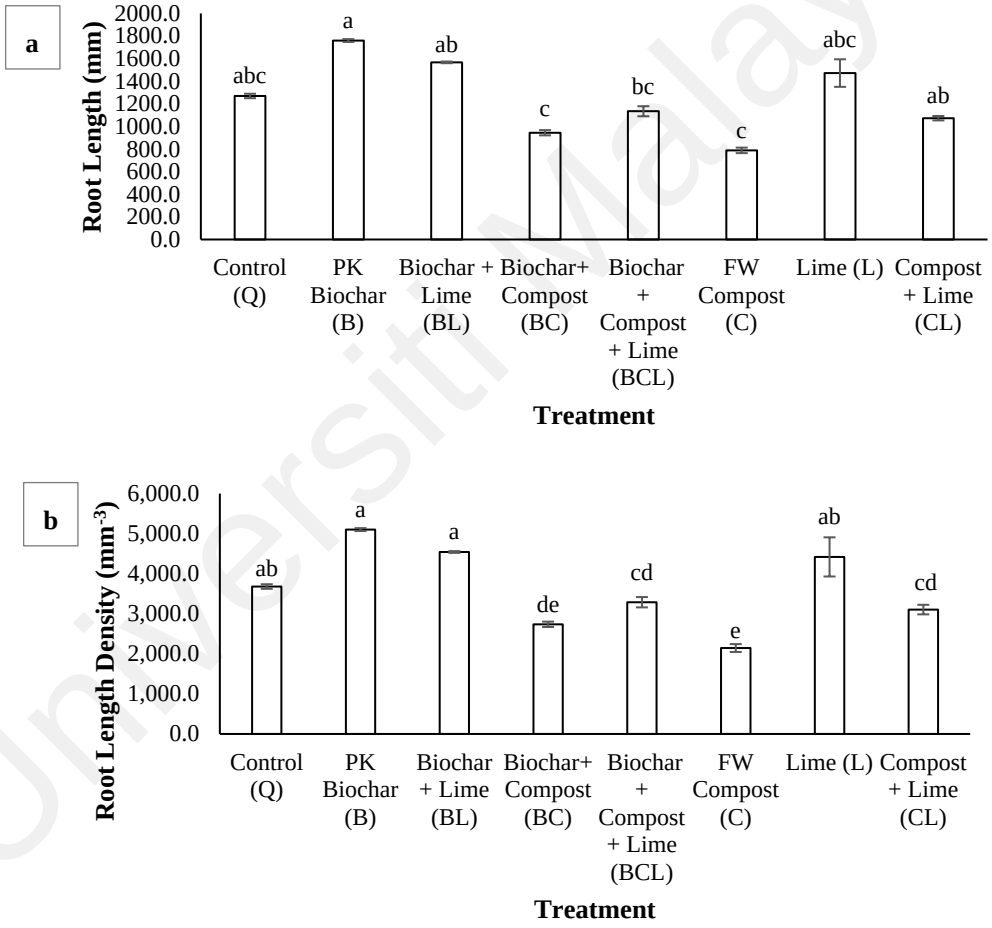


Figure 4.4: Effects of different amendments on (a) root length, (b) root length density, (c) root average diameter and (d) root volume of *M. malabathricum* L. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatments during final planting at $p \leq 0.05$.

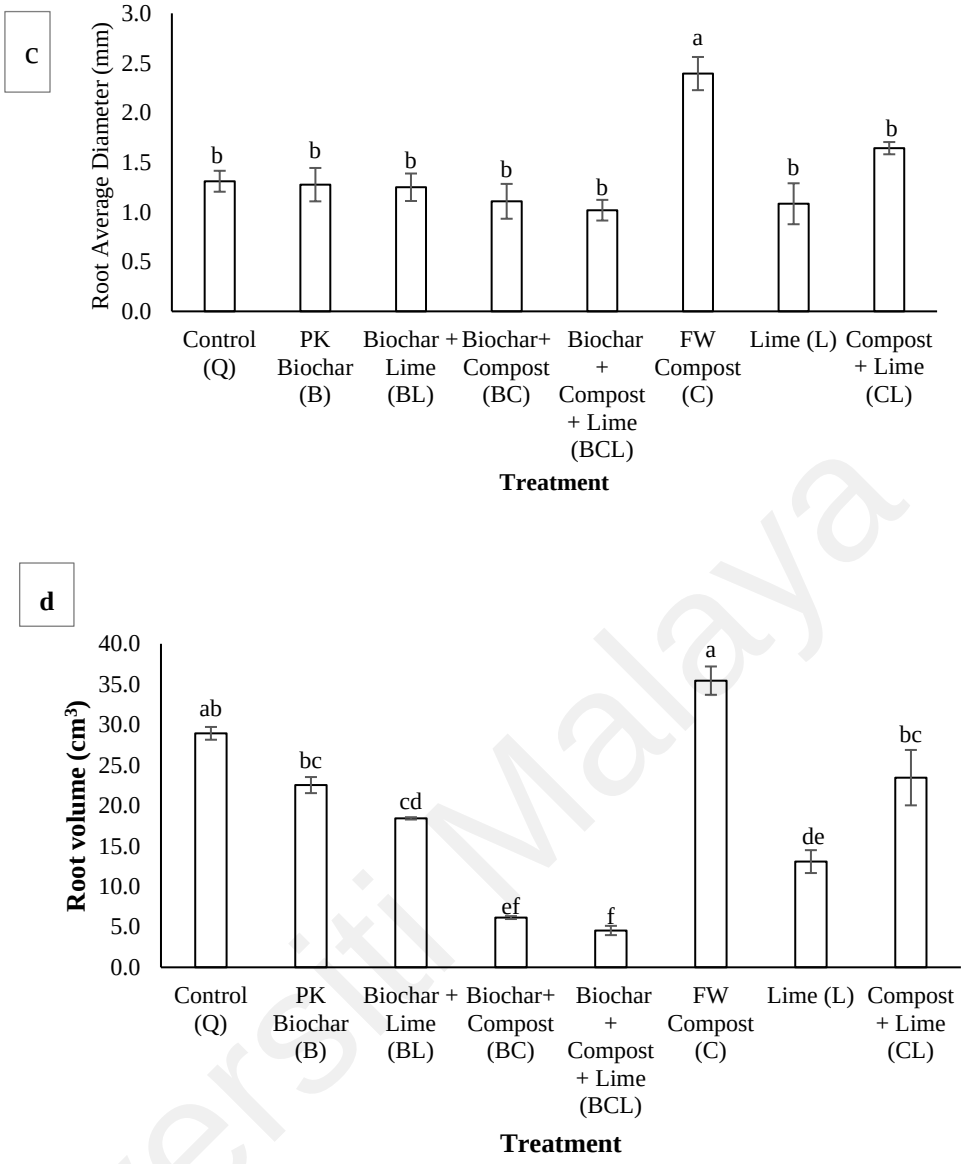


Figure 4.4, continued.

4.1.4 Plant Biomass

Generally, significant differences among treatments were observed in the leaf, stem and root biomass (Figure 4.5). The application of C exhibited the highest leaf biomass, which was 22.37% higher than the control. Moreover, B treatment recorded the highest stem biomass, which was 16.77% higher than the control (Q). BL treatment displayed the highest root biomass, which was 67.73% significantly higher than those in the control. Different treatments responded differently in the development of dry matter.

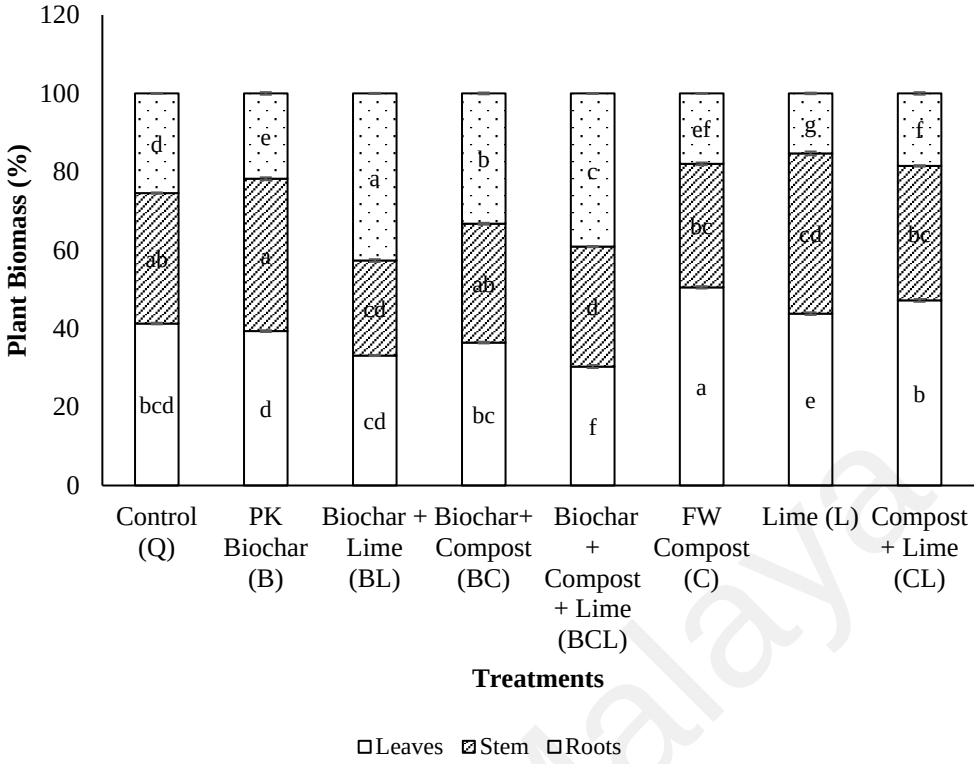


Figure 4.5: Plant biomass in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatments during final planting at $p \leq 0.05$.

4.1.5 Plant Nutrient Content

From this study, there was an increase in plant nutrient uptake in the soil treated with soil amendments compared to the control (Table 4.1). *M. malabathricum* L. grown in acidic soil treated with L treatment (58.60%) exhibited the highest N content followed by B (45.53%) and C treatments (42.28%) compared to the control (Q). Meanwhile, the highest available P content on *M. malabathricum* L. was recorded in BC treatment at 89.02% higher than the control plant followed by C treatment with 86.58%. BL treatment showed the highest exchangeable K content followed by BCL and BC, with 86.91%, 74.79% and 47.13%, respectively compared to the control.

Table 4.1: Effects of different soil amendments on plant nutrient content.

Treatments	Total N	Available P	Exchangeable K
	%	mg/kg	meq/100g
Control (Q)	1.017 ± 0.038[b]	0.082 ± 0.001[f]	0.940 ± 0.006[e]
PK Biochar (B)	1.480 ± 0.066[a]	0.142 ± 0.001[bc]	1.313 ± 0.017 [d]
Biochar + Lime (BL)	1.043 ± 0.047[a]	0.137 ± 0.002[c]	1.757 ± 0.026[a]
Biochar+ Compost (BC)	1.090 ± 0.061[b]	0.155 ± 0.001[a]	0.963 ± 0.015[e]
Biochar + Compost + Lime (BCL)	1.117 ± 0.032[b]	0.145 ± 0.003[b]	1.643 ± 0.009[b]
FW Compost (C)	1.447 ± 0.059[a]	0.153 ± 0.001[a]	1.553 ± 0.015[c]
Lime (L)	1.613 ± 0.052[a]	0.123 ± 0.002[d]	1.383 ± 0.003[d]
Compost + Lime (CL)	1.123 ± 0.037[b]	0.105 ± 0.001[e]	1.010 ± 0.012[e]

N: nitrogen; P: phosphorus; K: potassium; meq: milliequivalent. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

4.1.6 Soil and Soil Amendments Properties

The physicochemical properties of soil and soil amendments used in the study are shown in Table 4.2. Significantly lower soil pH was observed in treatment Q (control) (4.47 ± 0.03) at the final planting stage. Evidently, the control plant (Q) took longer to completely disintegrate and react with the soil to maintain its pH level (Table 4.3). The soil pH grown by *M. malabathricum* L. acidic soil treated with CL has increased by 48.16% followed by BL and BCL treatment by 44.42% and 34.36%, respectively compared to the control.

After harvest, the chemical characteristics of acidic soil were affected by the soil amendment applications as shown in Table 4.4 and Table 4.5. Based on the overall data analysis, soil amendments were found to influence the mineral composition of the soil. Significantly higher levels of macronutrients N, P and exchangeable cations of K, Ca, Mg and Na were reported in the biochar (B) and compost (C) treatment, whereas the control had the highest levels of exchangeable micronutrients (Zn, Fe, Mn, Cu, Cd and Al).

Exchangeable Ca and Na in the compost treatment were also found to be comparable with those in the biochar treatment. This study shows that exchangeable Zn, Fe and Mn in the soils can be significantly reduced by applying soil amendments.

Table 4.2: Physico-chemical properties of soil and soil amendments used in the study.

Properties	Soil	PK Biochar	FW Compost
pH	3.90 ± 0.058^{c}	8.61 ± 0.058^{a}	6.60 ± 0.006^{b}
EC (dS/M)	0.10 ± 0.058^{c}	3.67 ± 0.058^{a}	2.84 ± 0.006^{b}
Texture	Sandy loam	-	-
Total OC	3.97 ± 0.055^{c}	43.41 ± 0.107^{a}	14.34 ± 0.516^{b}
N (%)	0.06 ± 0.046^{c}	0.5 ± 0.028^{b}	2.39 ± 0.100^{a}
Available P (mg/kg)	0.29 ± 0.017^{b}	0.15 ± 0.050^{b}	2.82 ± 0.665^{a}
K (meq/100g)	0.11 ± 0.058^{b}	0.74 ± 0.202^{a}	$0.21 \pm 0..0065^{b}$
Ca (meq/100g)	1.20 ± 0.0065^{b}	2.27 ± 0.069^{a}	0.76 ± 0.058^{c}
Mg (meq/100g)	0.26 ± 0.127^{a}	0.25 ± 1.035^{a}	0.36 ± 0.006^{a}

EC: electrical conductivity; OC: organic carbon; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

Table 4.3: Effects of different soil amendments on soil physico-chemical properties after six months of the study.

Treatments	pH	EC	CEC	Moisture	OM	OC
		dS/M				
Control (Q)	4.473 ± 0.019^{d}	0.100 ± 0.033^{c}	8.957 ± 1.035^{b}	205.292 ± 9.982^{bcd}	207.073 ± 4.644^{bcd}	120.112 ± 2.694^{bcd}
PK Biochar (B)	5.260 ± 0.058^{c}	0.200 ± 0.000^{ab}	14.956 ± 1.013^{ab}	224.059 ± 12.530^{abc}	220.961 ± 11.965^{bc}	128.168 ± 6.940^{bc}
Biochar+Lime (BL)	6.460 ± 0.153^{a}	0.200 ± 0.000^{ab}	11.653 ± 0.374^{ab}	188.587 ± 8.297^{cd}	185.728 ± 8.756^{cd}	107.731 ± 5.079^{cd}
Biochar+ Compost (BC)	5.227 ± 0.152^{c}	0.200 ± 0.000^{ab}	14.175 ± 1.415^{ab}	254.284 ± 11.645^{ab}	250.776 ± 10.983^{ab}	145.462 ± 6.940^{ab}
Biochar+Compost+Lime (BCL)	6.010 ± 0.000^{ab}	0.267 ± 0.033^{a}	16.450 ± 4.460^{ab}	154.166 ± 18.039^{d}	152.475 ± 17.646^{d}	88.443 ± 10.236^{d}
FW Compost (C)	5.600 ± 0.031^{bc}	0.167 ± 0.033^{bc}	16.153 ± 0.609^{ab}	218.632 ± 19.389^{abcd}	215.967 ± 18.580^{bc}	125.271 ± 10.777^{bc}
Lime (L)	6.560 ± 0.199^{a}	0.100 ± 0.000^{c}	17.210 ± 0.640^{a}	179.051 ± 2.210^{cd}	177.509 ± 1.976^{cd}	102.963 ± 1.146^{cd}
Compost + Lime (CL)	6.627 ± 0.260^{a}	0.133 ± 0.033^{bc}	15.190 ± 3.130^{ab}	282.248 ± 0.707^{a}	279.420 ± 0.640^{a}	162.077 ± 0.371^{a}

EC: electrical conductivity; CEC: cation exchange capacity; OM: organic matter; OC: organic carbon. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

Table 4.4: The effects of different treatments on the macronutrients of soil.

Treatments	Total N	Available P	Exchangeable cation			
			K	Ca	Mg	Na
	%	mg/kg	meq/100g			
Control (Q)	0.033 ± 0.003^{e}	2.500 ± 0.5^{f}	0.153 ± 0.013^{c}	1.287 ± 0.009^{d}	0.287 ± 0.027^{c}	0.030 ± 0.0^{abc}
PK Biochar (B)	0.115 ± 0.005^{a}	79.500 ± 8.5^{b}	1.070 ± 0.13^{b}	1.370 ± 0.1^{cd}	0.213 ± 0.015^{c}	0.035 ± 0.005^{abc}
Biochar + Lime (BL)	0.047 ± 0.003^{de}	5.667 ± 0.667^{ef}	0.260 ± 0.015^{c}	2.217 ± 0.231^{abcd}	0.647 ± 0.043^{b}	0.033 ± 0.003^{abc}
Biochar+ Compost (BC)	0.070 ± 0.006^{cd}	38.500 ± 5.5^{cd}	0.233 ± 0.029^{c}	1.597 ± 0.143^{bcd}	0.233 ± 0.013^{c}	0.020 ± 0.0^{c}
Biochar + Compost + Lime (BCL)	0.050 ± 0.0^{cde}	17.500 ± 1.5^{ef}	0.150 ± 0.02^{c}	2.017 ± 0.187^{abcd}	0.547 ± 0.038^{b}	0.027 ± 0.003^{bc}
FW Compost (C)	0.123 ± 0.009^{a}	190.500 ± 1.5^{a}	1.700 ± 0.151^{a}	2.280 ± 0.142^{abc}	0.277 ± 0.020^{c}	0.040 ± 0.006^{ab}
Lime (L)	0.103 ± 0.003^{ab}	52.000 ± 6.0^{c}	1.363 ± 0.074^{ab}	2.697 ± 0.232^{a}	0.720 ± 0.059^{ab}	0.047 ± 0.003^{a}
Compost + Lime (CL)	0.077 ± 0.007^{bc}	25.333 ± 2.848^{de}	0.235 ± 0.015^{c}	2.545 ± 0.345^{ab}	0.857 ± 0.073^{a}	0.033 ± 0.0^{abc}

N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; Na: sodium; meq: milliequivalent. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

Table 4.5: The effects of different treatments on the micronutrients of soil.

Treatments	Micronutrient					
	Zn	Fe	Mn	Cu	Cd	Al
	mg/kg					
Control (Q)	2.653 ± 0.018^{b}	191.270 ± 5.048^{a}	2.653 ± 0.034^{b}	31.090 ± 0.516^{b}	0.793 ± 0.054^{b}	0.021 ± 0.001^{a}
PK Biochar (B)	1.927 ± 0.067^{c}	77.227 ± 1.998^{d}	1.783 ± 0.107^{c}	4.273 ± 0.069^{d}	0.723 ± 0.049^{c}	0.020 ± 0.0^{ab}
Biochar+Lime (BL)	1.247 ± 0.046^{d}	70.307 ± 3.534^{d}	2.553 ± 0.123^{b}	4.490 ± 0.100^{d}	0.820 ± 0.026^{b}	0.019 ± 0.0^{bc}
Biochar+ Compost (BC)	2.270 ± 0.055^{c}	190.890 ± 16.075^{a}	2.290 ± 0.029^{bc}	18.010 ± 1.67^{c}	0.790 ± 0.040^{b}	0.020 ± 0.0^{ab}
Biochar+Compost+Lime (BCL)	1.423 ± 0.127^{d}	64.185 ± 0.665^{d}	2.467 ± 0.221^{b}	2.753 ± 0.263^{d}	1.165 ± 0.015^{a}	0.019 ± 0.0^{abc}
FW Compost (C)	2.140 ± 0.047^{c}	133.747 ± 2.139^{b}	2.340 ± 0.050^{bc}	4.613 ± 0.178^{d}	0.670 ± 0.072^{bc}	0.020 ± 0.0^{abc}
Lime (L)	1.273 ± 0.032^{d}	111.633 ± 3.576^{bc}	2.633 ± 0.050^{b}	3.307 ± 0.105^{d}	0.495 ± 0.065^{c}	0.019 ± 0.0^{abc}
Compost + Lime (CL)	5.377 ± 0.138^{a}	87.570 ± 0.963^{cd}	8.467 ± 0.202^{a}	36.237 ± 1.680^{a}	0.913 ± 0.068^{ab}	0.018 ± 0.0^{c}

Zn: zink; Fe: ferum; Mn: manganese; Cu: copper; Cd: cadmium; Al: aluminium. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

4.2 The Physiological Characteristics of *M. malabathricum* L. on Acidic Conditions.

4.2.1 Relative Chlorophyll Content

Generally, treatments C, B and BL recorded significantly higher relative chlorophyll content. The relative chlorophyll content of *M. malabathricum* L. grown in treatment C showed the highest value followed by treatment C and BL (Figure 4.6). There was a significant increment of 16.20%, 15.72% and 15.18% in treatments C, B and BL compared to the control.

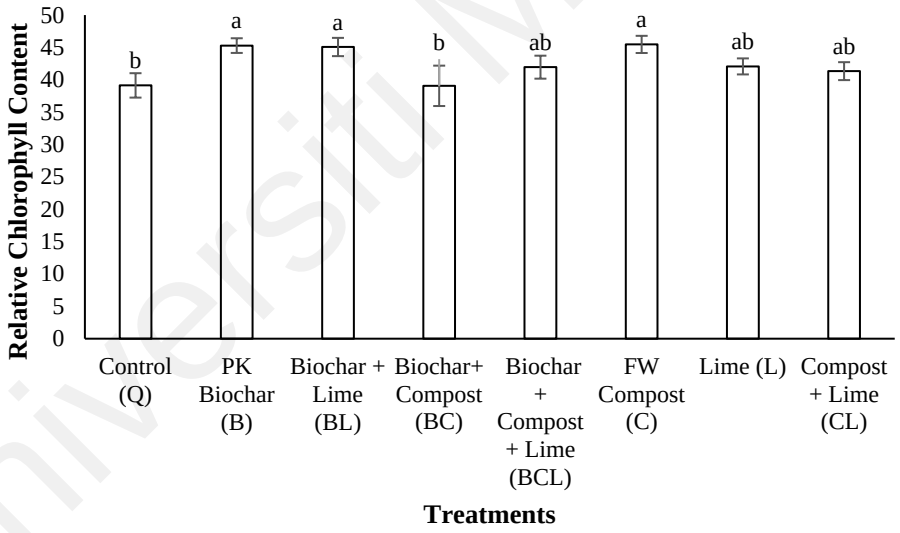


Figure 4.6: Relative chlorophyll content in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatments at $p \leq 0.05$.

4.2.2 Rate of Photosynthesis, Stomatal Conductance, Transpiration Rate and Water Use Efficiency

In general, all treatments resulted in positive responses in the physiological performance of *M. malabathricum* L. (Figure 4.7). The photosynthetic rates of all

treatments were significantly higher compared to the control (Q). In the final planting period, a significantly higher rate of photosynthesis was observed in treatment C (10.646 µmol m^{-2} s^{-1}**)** followed by BL treatment (10.122 µmol m^{-2} s^{-1}). Based on the results, combined treatment CL, L and C were found to be significantly higher in stomatal conductance with 0.059 mol m^{-2} s^{-1}, 0.056 mol m^{-2} $s^{-1}$and 0.049 mol m^{-2} s^{-1}, respectively compared to other treatments. All treatments recorded significantly higher transpiration rates compared to the control, excluding BCL. The highest transpiration rate was exhibited in combined treatment CL (2.053 mmol m^{-2} s^{-1}**)** followed by L (1.880 mmol m^{-2} s^{-1}) and C (1.649 mmol m^{-2} s^{-1}). However, as for water use efficiency, a significantly higher water use efficiency was observed in BCL treatment followed by BL and the control plant.

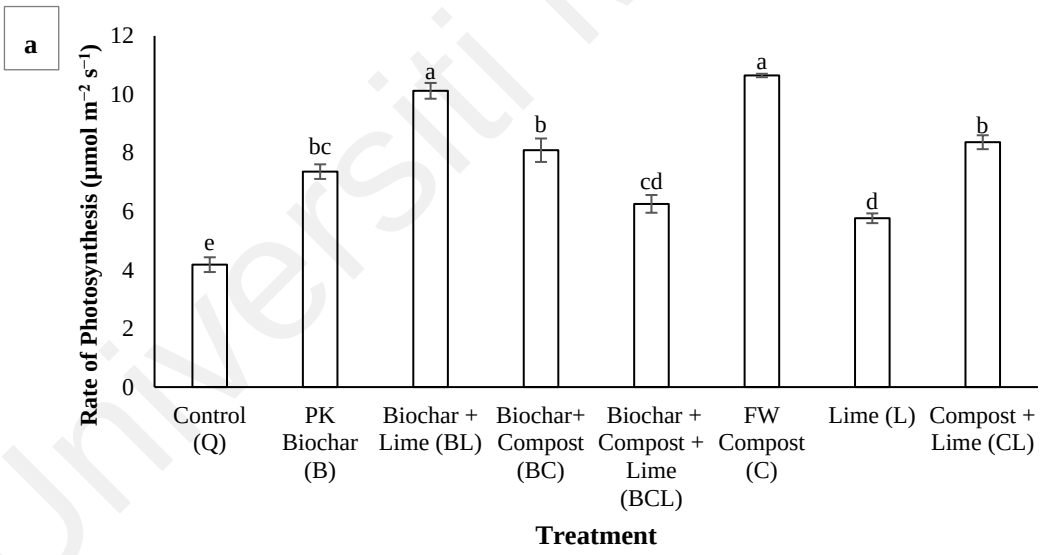
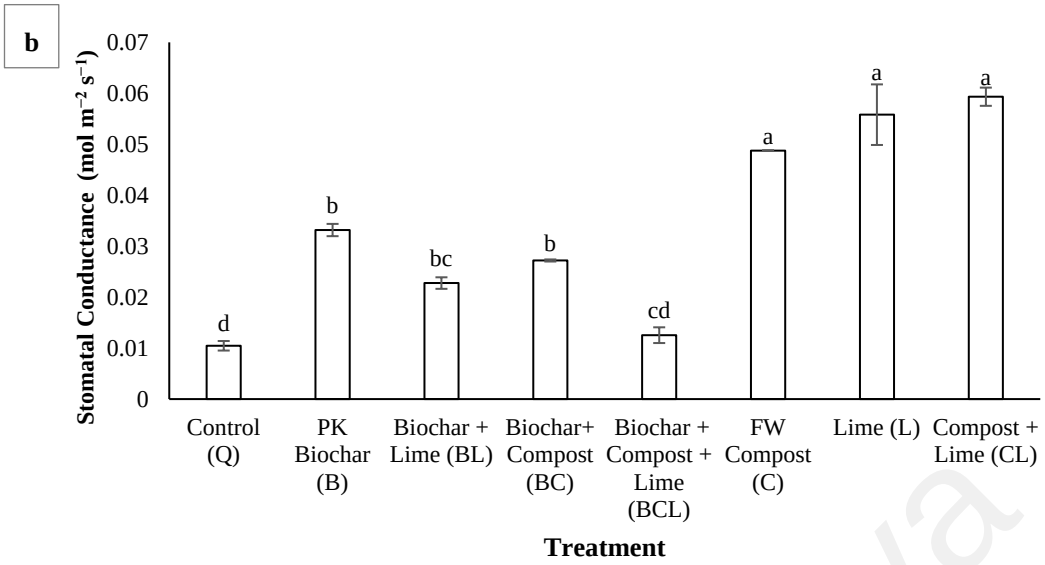
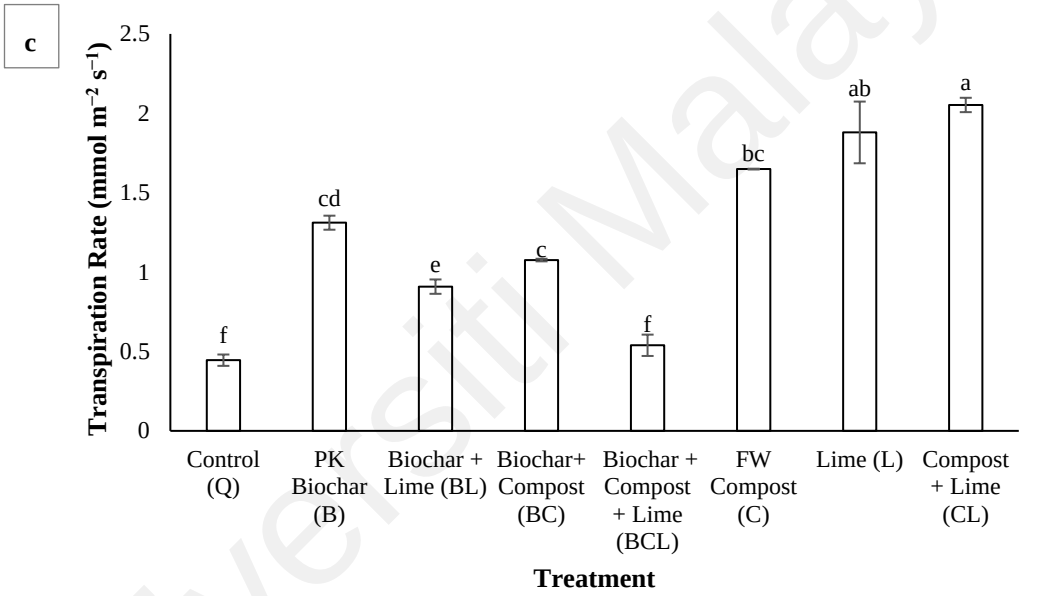


Figure 4.7: Rate of photosynthesis (a), stomatal conductance (b), transpiration rate (c) and water use efficiency (d) in different soil amendments. Vertical bars represent standard error. Different letters indicate a significant difference amongst the treatments at $p \leq 0.05$.





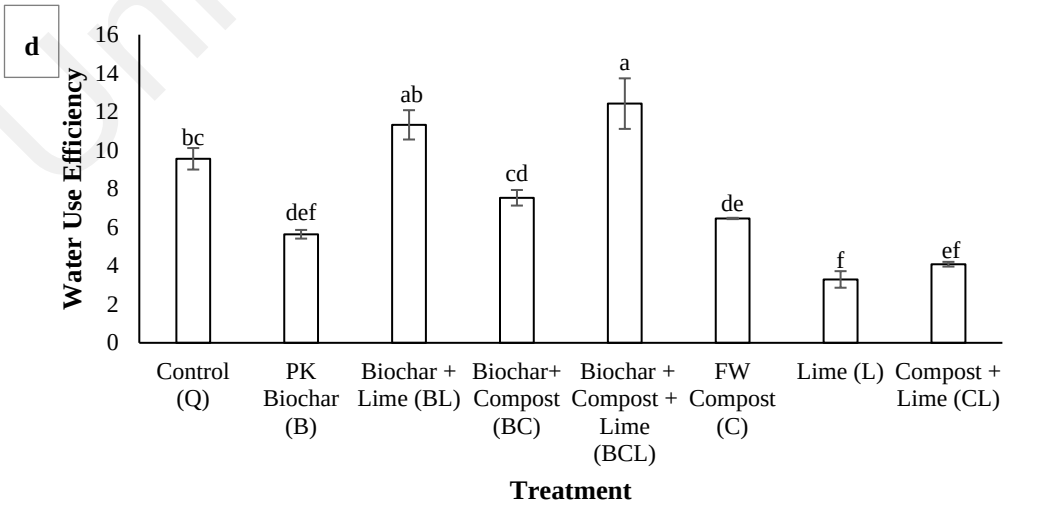


Figure 4.7. Continued

4.2.3 Correlation Between Growth and Nutrient Uptake

The correlations table between parameters related to growth, plant physiological characteristics and nutrient uptake of *M. malabathricum* L. grown with different amendments in acidic soil conditions is shown in Table 4.6. The results indicate that there is a strong negative correlation between root length and transpiration rate ($r=-0.906$, $p \leq 0.01$), stomatal conductance ($r=-0.802$, $p \leq 0.05$) and Ca ($r=-0.764$, $p \leq 0.05$). Stomatal conductance indicated a strong positive correlation with transpiration rate ($r=0.953$, $p \leq 0.01$) and Ca ($r=0.724$, $p \leq 0.05$). Element Ca also recorded a positive correlation with transpiration rate ($r=0.841$, $p \leq 0.01$), soil pH ($r=0.765$, $p \leq 0.05$) and EC ($r=0.871$, $p \leq 0.01$). A strong positive correlation was also exhibited between the available P of the soil with Total N ($r=0.844$, $p \leq 0.01$) and Total P ($r=0.937$, $p \leq 0.01$). Al, shows a strong negative correlation (with $p \leq 0.01$) with element K ($r=-0.839$, $p \leq 0.01$) and Ca ($r=-0.869$, $p \leq 0.01$).

Table 4.6: Significant Pearson's Correlation between Parameters.

		Growth										Plant physiology				Soil nutrient															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Height	1																													
2	Stem	0.619	1																												
3	Root length	0.331	0.15	1																											
4	Root diameter	0.029	0.234	-0.559	1																										
5	Root volume	0.031	0.382	-0.107	.804*	1																									
6	RLD	0.426	-0.038	0.359	-0.143	0.037	1																								
7	Leaf biomass	-0.131	0.051	-0.324	0.05	0.025	0.392	1																							
8	Stem biomass	0.39	.913**	0.285	0.11	0.426	0.035	0.249	1																						
9	Root biomass	0.052	-0.222	0.641	-0.419	-0.296	-0.05	-0.282	-0.109	1																					
10	LAI	0.504	0.102	0.593	-.842**	-.736*	0.312	-0.113	0.06	0.374	1																				
11	SPAD	-0.285	-0.2	-0.609	-0.019	-0.123	-0.191	0.202	-0.243	-0.592	-0.116	1																			
12	Photosynthesis	0.465	0.111	-0.146	0.486	0.227	-0.12	-0.241	-0.107	0.322	-0.146	-0.174	1																		
13	Stomata	0.192	0.137	-.802*	0.533	0.179	0.112	0.492	-0.04	-0.682	-0.313	0.574	0.302	1																	
14	Transpiration	-0.059	-0.109	-.906**	0.566	0.155	0.041	0.465	-0.26	-0.692	-0.466	0.591	0.222	.953**	1																
15	pH	0.046	-0.689	-0.225	-0.074	-0.444	0.256	0.012	-.816*	0.244	0.145	0.111	0.445	0.276	0.371	1															
16	EC	-0.093	-0.347	-0.646	0.535	0.273	0.092	0.299	-0.415	-0.246	-0.49	0.502	0.528	.727*	.780*	0.58	1														
17	CEC	0.252	-0.094	-0.528	0.082	-0.376	-0.132	-0.254	-0.455	-0.467	0.144	0.444	0.191	0.539	0.551	0.441	0.245	1													
18	TotalN	0.523	0.571	-0.454	0.428	0.271	-0.082	-0.017	0.294	-0.643	-0.073	0.503	0.329	.726*	0.557	-0.131	0.334	0.58	1												
19	TotalP	0.141	0.395	-0.51	.801*	0.601	-0.538	-0.289	0.173	-0.308	-0.6	0.175	0.612	0.442	0.4	-0.175	0.402	0.267	0.66	1											
20	AvailableP	0.308	0.541	-0.427	0.649	0.52	-0.445	-0.32	0.283	-0.448	-0.387	0.311	0.483	0.475	0.368	-0.278	0.289	0.385	.844**	.937**	1										
21	K	0.219	0.365	-0.462	0.421	0.379	-0.235	-0.138	0.167	-0.626	-0.249	0.689	0.24	0.599	0.491	-0.186	0.426	0.445	.899**	0.702	.864**	1									
22	Ca	-0.181	-0.548	-.764*	0.332	-0.116	0.018	0.227	-0.694	-0.315	-0.321	0.562	0.36	.724*	.841**	.765*	.871**	0.578	0.262	0.225	0.133	0.288	1								

Table 4.6, Continued

		Growth											Plant physiology				Soil nutrient														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
23	Mg	-0.232	-.744*	-0.434	0.033	-0.282	0.412	0.384	-.756*	-0.134	-0.107	0.293	0.062	0.447	0.602	.837**	0.649	0.337	-0.164	-0.3	-0.399	-0.179	.831*	1							
24	Na	0.025	-0.036	-0.371	0.265	0.338	0.162	0.053	-0.104	-0.622	-0.24	.784*	0.019	0.559	0.525	0.049	0.594	0.304	0.625	0.319	0.474	.820*	0.45	0.218	1						
25	Al	-0.147	0.488	0.438	-0.242	0.203	-0.352	-0.207	0.656	0.045	0.07	-0.097	-0.481	-0.558	-0.637	-.909**	-0.689	-0.506	-0.078	0	0.111	0.069	-.839**	-.869**	-0.118	1					
26	Zn	-0.004	0.129	-0.401	0.546	0.3	0.27	0.59	0.139	-0.324	-0.446	-0.315	0.02	0.425	0.481	0.03	0.175	-0.037	-0.027	0.061	-0.095	-0.275	0.198	0.316	-0.273	-0.382	1				
27	Fe	-0.463	0.34	-0.112	0.027	0.17	-0.57	0.297	0.556	-0.006	-0.287	0.011	-0.325	-0.173	-0.176	-0.7	-0.312	-0.514	-0.143	0.131	0.06	-0.056	-0.427	-0.541	-0.295	0.678	0.095	1			
28	Mn	-0.064	-0.212	-0.495	0.45	0.103	0.38	0.59	-0.23	-0.288	-0.379	-0.142	0.092	0.526	0.633	0.412	0.419	0.147	-0.076	-0.058	-0.224	-0.289	0.521	0.675	-0.135	-0.698	.911**	-0.191	1		
29	Cu	-0.334	0.035	-0.181	0.281	0.248	0.175	0.666	0.242	-0.126	-0.422	-0.367	-0.303	0.066	0.159	-0.208	-0.068	-0.442	-0.394	-0.202	-0.382	-0.519	-0.088	0.157	-0.435	-0.029	.857**	0.442	.708*	1	
30	Cd	-0.015	-0.326	0.328	-0.131	-0.286	0.03	-0.426	-0.391	0.466	0.137	-.731*	0.038	-0.532	-0.397	0.29	-0.41	0.048	-0.596	-0.29	-0.434	-.724*	-0.16	0.112	-.724*	-0.215	0.205	-0.296	0.237	0.183	1
** Correlation is significant at the 0.01 level (2-tailed).																															
* Correlation is significant at the 0.05 level (2-tailed).																															

1: height; 2: stem; 3: root length; 4: root diameter; 5: 6: RLD; 7: leaf biomass; 8: stem biomass; 9: root biomass; 10: LAI; 11: SPAD; 12: photosynthesis; 13: stomata; 14: transpiration; 15: pH; 16: EC; 17: CEC; 18: Total N; 19: Total P; 20: Available P; 21: K; 22: Ca; 23: Mg; 24: Na; 25: Al; 26: Zn; 27: Fe; 28: Mn; 29: Cu; 30: Cd; RLD: root length density; LAI: leaf area index; EC: electrical conductivity; CEC: cation exchange capacity; N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; Na: sodium; Al: aluminium; Zn: zink; Fe: ferum; Mn: manganese; Cu: copper; Cd: cadmium

4.3 Cumulative Ranking Analysis of Growth, Nutrient Uptake and Physiological Characteristics

This study provided information on plant growth performance, nutrient uptake and plant physiological characteristics of *M. malabathricum* L. grown with different amendments in acidic soil conditions (Table 4.7). Plant growth performances such as plant height, stem diameter, leaf area index, root profiles (root length, root length density, root average diameter and root volume) and plant biomass (leaf, stem and root) were evaluated. Moreover, the nutrient uptake for both plants and soil were included in the evaluation. In addition, the plant's physiological characteristics, including the rate of photosynthesis, stomatal conductance, transpiration rate, and water use efficiency, were also taken into consideration. This analysis showed that the plants grown with PK biochar (treatment B) were ranked best followed by the plant grown with FW compost, treatment C. Thus, these two treatments were chosen for subsequent analysis in this research.

Table 4.7: Cumulative ranking analysis of the parameters studied in different soil treatment

Parameter/ Treatment	Control (Q)	PK Biochar (B)	Biochar + Lime (BL)	Biochar + Compost (BC)	Biochar+ Compost + Lime (BCL)	FW Compost (C)	Lime (L)	Compost + Lime (CL)
Plant height	1	8	7	6	3	4	2	5
Stem diameter	5	8	2	7	1	6	3	4
Leaf area index	1	8	5	7	6	3	4	2
Root length	5	8	7	2	4	1	6	3
Root length density	5	8	7	2	4	1	6	3
Root average diameter	6	5	4	3	1	8	2	7
Root volume	7	5	4	2	1	8	3	6
Leaf biomass	5	4	2	3	1	8	6	7
Stem biomass	5	7	1	2	3	4	8	6
Root biomass	5	4	8	6	7	2	1	3
Plant nutrient uptake	2	6	4	4	5	8	7	3
Soil physico-chemical	1	7	4	6	2	5	3	8
Soil nutrient uptake (macronutrient)	2	5	4	3	3	7	8	6
Soil nutrient uptake (micronutrient)	1	8	5	3	6	4	7	2
Relative chlorophyll content	2	7	6	1	4	8	5	3
Rate of photosynthesis	1	4	7	5	3	8	2	6
Stomatal conductance	1	5	3	4	2	6	7	8
Transpiration rate	1	5	3	4	2	6	7	8
Water use efficiency	6	3	7	5	8	4	1	2
Total value	62	115	90	75	66	101	88	92
Ranking		1st				2nd		

A PCA analysis was conducted to investigate the plant growth, plant physiological characteristics and soil nutrient traits distribution (Fig. 4.8). Biplot was attained using two main components (PC1-2) whereby 64.9% of the total variance was explained by the first component whilst the second major component justified 35.0% of the total variation. The first component was correlated to root volume, root diameter, relative chlorophyll content, EC, transpiration rate, available P and photosynthesis rate. There is a close relation between FW compost treatments and the first component indicating the importance of these parameters in the improvement of *M. malabathricum* L. grown in acidic soil conditions.

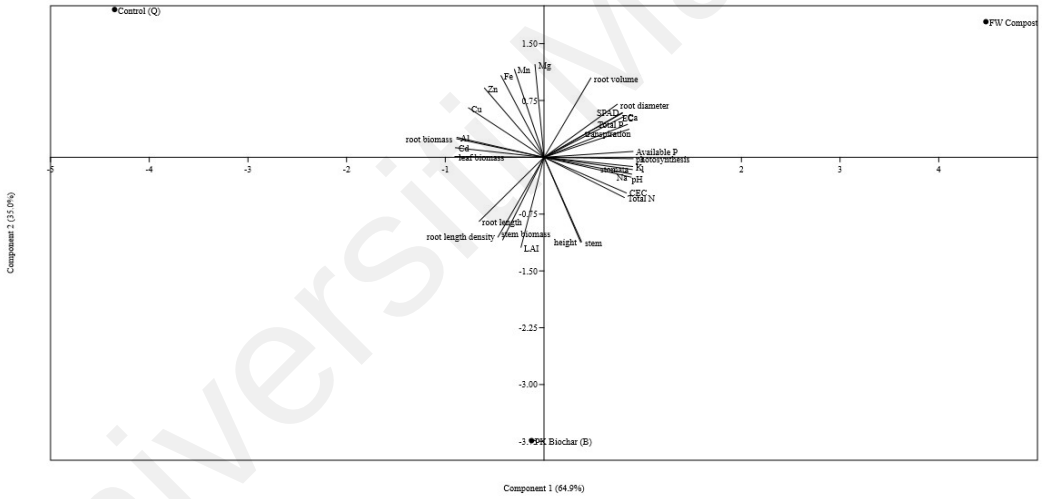


Figure 4.8: Principal component analysis (PCA) of plant growth, plant physiological characteristics and soil nutrient parameters of *M. malabathricum* L. treated with the best two treatments.

4.4 Effects of Different Soil amendment Approaches on Plant Cellular Antioxidants and Oxidative Stress Indicators.

4.4.1 Oxidative Stress Indicators

4.4.1.1 Hydrogen Peroxide (H_2O_2)

The application of treatments C and B significantly ($p \leq 0.05$) improved the in vivo H_2O_2 levels in the plants grown with both amendments (Table 4.8). The H_2O_2 level in the leaves of the plants subjected to the control treatment was significantly higher (860.58 ± 25.70 µmole g^{-1} FW) compared to the plants grown with treatments B and C. A similar trend was observed in the stem and root extracts where treatment Q (control) recorded significantly higher H_2O_2 levels with 692.024 ± 50.24 µmole g^{-1} FW and 186.361 ± 11.76 µmole g^{-1} FW, respectively. The lowest levels of H_2O_2 were observed in the extracts of both leaves and roots in B treatments, while the lowest levels of H_2O_2 in the stem were observed in treatment C.

Table 4.8: Effects of different treatments on hydrogen peroxide content in various parts of *M. malabathricum* L. methanolic extracts.

	H_2O_2 (µmole g^{-1} FW)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	860.579 ± 25.70a	692.024 ± 50.24a	186.361 ± 11.76a
PK Biochar (B)	200.080 ± 23.11c	398.034 ± 23.76b	74.644 ± 15.56b
FW Compost(C)	441.152 ± 3.92b	256.918 ±11.76b	96.203 ± 3.92b

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; H_2O_2: hydrogen peroxide; FW: fresh weigh.

4.4.2 Antioxidant Enzyme Activities

4.4.2.1 Superoxide Dismutase (SOD)

The results showed that the SOD activity was significantly higher in the leaves of plants grown with C (110.0 ± 1.25 units mg^{-1} DW) compared to those in the control, Q (76.25 ± 7.50 units mg^{-1} DW) (Table 4.9). A similar trend was observed in the root samples. Comparatively higher SOD activity was observed in the stem of plants grown with C (73.13 ± 3.13 units mg^{-1} DW) followed by treatments B (71.25 ± 2.50 units mg^{-1} DW) and Q (66.25 ± 2.50 units mg^{-1} DW).

Table 4.9: Effects of different treatments on superoxide dismutase (SOD) activity in various parts of *M. malabathricum* L.

	SOD activity (units mg^{-1} DW)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	76.25 ± 7.50^{b}	66.25 ± 2.50^{a}	70.63 ± 0.63^{b}
PK Biochar (B)	89.38 ± 3.13^{ab}	71.25 ± 2.50^{a}	76.25 ± 1.25^{b}
FW Compost(C)	110.0 ± 1.25^{a}	73.13 ± 3.13^{a}	87.5 ± 1.25^{a}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; SOD: superoxide dismutase; DW: dry weight.

4.4.2.2 Catalase (CAT)

The CAT activity was observed to be significantly higher in the leaf samples from plants grown with Q (146 ± 2.0 µmol min^{-1} mg^{-1} DW) compared to B (50 ± 2.0 µmol min^{-1} mg^{-1} DW) and C (48 ± 0.0 µmol min^{-1} mg^{-1} DW) (Table 4.10). In the stem extracts, a similar trend was observed among the three treatments, however, no significant difference was observed. In addition, a significantly higher CAT activity level was found in the root samples from plants grown with Q (28 ± 0.0 µmol min^{-1} mg^{-1} DW) followed by B (20 ± 4.0 µmol min^{-1} mg^{-1} DW) and C (12 ± 0.0 µmol min^{-1} mg^{-1} DW).

Table 4.10: Effects of different treatments on catalase (CAT) activity in various parts of *M. malabathricum* L.

	CAT activity (µmol min^{-1} mg^{-1} DW)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	146 ± 2.00^{a}	32 ± 4.00^{a}	28 ± 0.00^{a}
PK Biochar (B)	50 ± 2.00^{b}	28 ± 4.00^{a}	20 ± 4.00^{ab}
FW Compost(C)	48 ± 0.00^{b}	22 ± 2.00^{a}	12 ± 0.00^{b}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; CAT: catalase; DW: dry weight.

4.4.2.3 Ascorbate Peroxidase (APX)

The comparison between treatments revealed that there was no significant difference in the APX activity exhibited by the leaf extract (Table 4.11). A similar trend was also observed in the root extracts. The APX activity of treatment C (2.60 ± 1.84 nmole min^{-1} mg^{-1} DW) was found to be higher than B (0.56 ± 0.40 nmole min^{-1} mg^{-1} DW) and Q (0.20 ± 0.16 nmole min^{-1} mg^{-1} DW). In the stem extract, a significantly higher APX activity level was observed in C (1.40 ± 0.28 nmole min^{-1} mg^{-1} DW).

Table 4.11: Effects of different treatments on ascorbate peroxidase (APX) activity in various parts of *M. malabathricum* L.

	APX activity (nmole min^{-1} mg^{-1} DW)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	1.04 ± 0.20^{a}	0.08 ± 0.04^{b}	0.20 ± 0.16^{a}
PK Biochar (B)	3.30 ± 2.38^{a}	0.18 ± 0.06^{b}	0.56 ± 0.40^{a}
FW Compost(C)	3.48 ± 2.24^{a}	1.40 ± 0.28^{a}	2.60 ± 1.84^{a}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; APX: ascorbate peroxidase; DW: dry weight.

4.4.3 Non-Enzymatic antioxidants

4.4.3.1 Chlorophyll Content

M. malabathricum L. leaf pigments content was significantly influenced by soil amendments (Table 4.12). A similar trend was observed between chlorophyll a and chlorophyll b contents. A significantly higher amount of total chlorophyll was obtained in treatment C (433.678 ± 13.224 µg g^{-1} DW) followed by B (356.845 ± 8.576 µg g^{-1} DW) and Q (273.252 ± 19.720 µg g^{-1} DW). The highest weight ratio of chlorophyll a to chlorophyll b (C*a*/C*b* ratio) recorded in treatment B was 2.756 ± 0.011 followed by C and Q with C*a*/C*b* ratios of 2.007 ± 0.025 and 2.010, respectively.

Table 4.12: Chlorophyll contents from methanolic extract of *M. malabathricum* L. leaves.

Treatment	C*a* (µg g^{-1} DW)	C*b* (µg g^{-1} DW)	C*a*+C*b* (µg g^{-1} DW)	C*a*/C*b* ratio
Control (Q)	182.623 ± 14.475^{b}	90.628 ± 5.298^{b}	273.252 ± 19.720^{c}	2.010 ± 0.049^{b}
PK Biochar (B)	261.837 ± 6.519^{a}	95.008 ± 2.069^{b}	356.845 ± 8.576^{b}	2.756 ± 0.011^{a}
FW Compost(C)	289.441 ± 8.881^{a}	144.237 ± 4.585^{a}	433.678 ± 13.224^{a}	2.007 ± 0.025^{b}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; Ca: chlorophyll a; C*b*: chlorophyll b; C*a*+C*b*: total chlorophyll content; C*a*/C*b* ratio: Chlorophyll a and b ratio; DW: dry weigh.

4.4.3.2 Carotenoid Content

The carotenoid contents were observed to be significantly higher in the leaves grown with treatment C (237.733 ± 7.22 µg g^{-1} DW) and treatment B (224.698 ± 5.77 µg g^{-1} DW) compared to the control (Table 4.13). In stem, a similar trend was observed. The carotenoid contents were observed to be significantly higher in treatment C (4.467 ± 0.15 µg g^{-1} DW) followed by treatment B (3.567 ± 0.98 µg g^{-1} DW). However, no significant

difference was observed in the root extract among the treatments.

Table 4.13: Carotenoid contents from methanolic extract of *M. malabathricum* L. leaves.

	***C(x+c)* (µg g^{-1} DW)**		
Treatment	**Leaves**	**Stem**	**Roots**
Control (Q)	189.936 ± 2.40^{b}	1.345 ± 0.14^{b}	0.557 ± 0.31^{a}
PK Biochar (B)	224.698 ± 5.77^{a}	3.567 ± 0.98^{ab}	0.645 ± 0.11^{a}
FW Compost(C)	237.733 ± 7.22^{a}	4.467 ± 0.15^{a}	0.720 ± 0.03^{a}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; *C*(*x*+*c*): carotenoid content; DW: dry weigh.

4.4.3.3 Proline Content

The free proline content analysis supported the hypothesis whereby the free proline content in the control treatment was significantly higher (4721.841 ± 16.81 µmole g^{-1} FW) compared to the plants subjected to soil amendment treatments (B and C) (Table 4.14). A similar trend was observed in the root extract with significantly highest value in Q (6225.455 ± 41.33 µmole g^{-1} FW) followed by treatment C (3005.520 ± 29.11 µmole g^{-1} FW) and B (2393.072 ± 51.34 µmole g^{-1} FW). Proline content in the stem samples grown with the control treatment was also found to be the highest (3137.545 ± 22.00 µmole g^{-1} FW).

Table 4.14: The effects of different treatments on Free Proline of the various parts of *M. malabathricum* L.

	Proline (µmole g^{-1} FW)		
Treatment	**Leaves**	**Stem**	**Roots**
Control (Q)	4721.841 ± 16.81^{a}	3137.545 ± 22.00^{a}	6225.455 ± 41.33^{a}
PK Biochar (B)	2734.136 ± 56.93^{c}	2745.138 ± 36.67^{b}	2393.072 ± 51.34^{c}
FW Compost(C)	3507.947 ± 34.98^{b}	1853.972 ± 14.67^{c}	3005.520 ± 29.11^{b}

FW: fresh weight; The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

4.4.3.4 Ascorbic Acid

The comparison between treatments showed no significant difference in the extract of all parts of *M. malabathricum* L. for ascorbic acid content (Table 4.15). A similar trend was observed in all parts whereby treatment C recorded the highest ascorbic acid in the leaves, stem and roots with 6.155 ± 0.02 µg mg^{-1} DW, 5.667 ±0.33 µg mg^{-1} DW and 5.715 ± 0.00 µg mg^{-1} DW, respectively. The lowest ascorbic acid content was detected in treatment Q at all parts of the plant studied.

Table 4.15: The effects of different treatments on Ascorbic Acid of the various parts of *M. malabathricum* L.

	Ascorbic Acid (µg mg^{-1} DW)		
Treatment	**Leaves**	**Stem**	**Roots**
Control (Q)	5.1271 ± 0.76[a]	4.895 ± 0.21[a]	5.064 ± 0.27[a]
PK Biochar (B)	6.032 ± 0.08[a]	5.424 ± 0.23[a]	5.388 ± 0.08[a]
FW Compost(C)	6.155 ± 0.02[a]	5.667 ±0.33[a]	5.715 ± 0.00[a]

DW: dry weight; The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$.

4.4.4 Secondary Metabolites Production

4.4.4.1 Phytochemical Screening

The methanolic extracts from different plant parts of *M. malabathricum* L. were analyzed, and data analysis revealed the presence of flavonoids and phenols in all samples (Table 4.16). However, no presence of phlobatannins and saponins was detected in all samples. Meanwhile, tannins were observed to be present in the leaves and root extract while alkaloid was only present in the leaves extract.

Table 4.16: Phytochemical screening of extracts from various parts of *M. malabathricum* L.

Phytochemical screening	Leaves			Stem			Roots		
	Control (Q)	PK Biochar (B)	FW Compost (C)	Control (Q)	PK Biochar (B)	FW Compost (C)	Control (Q)	PK Biochar (B)	FW Compost (C)
Alkaloids I	-	-	-	-	-	-	-	-	-
Alkaloids II	+	+	+	-	-	-	-	-	-
Alkaloids III	-	-	-	-	-	-	-	-	-
Flavonoids I	++	++	++	+	+	+	+	++	++
Flavonoid II	+++	+++	+++	+	+	++	+	++	++
Phenol	++	++	++	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-
Tannins	++	++	++	-	-	-	+	+	+

(+++) indicates appreciable amount of phytochemical; (++) indicate moderate amount of phytochemical; (+) indicate trace amount of phytochemical and (-) indicates the absence of the phytochemical; Q: control; B: palm kernel biochar; C: food waste compost.

4.4.4.2 Total Anthocyanin Content (TAC)

Based on Table 4.17, in the leaf samples, the highest total anthocyanin content (TAC) value was obtained in treatment C (0.36 ± 0.03 mg g^{-1} DW) followed by B (0.27 ± 0.04 mg g^{-1} DW) and Q (0.10 ± 0.04 mg g^{-1} DW). Data analysis further revealed that the differences observed in both treatments C and B were statistically significant compared to Q. The root also exhibited the highest TAC in C (0.09 ± 0.02 mg g^{-1} DW) followed by B (0.06 ± 0.03 mg g^{-1} DW) and Q (0.02 ± 0.00 mg g^{-1} DW). However, the TAC was observed to be the highest in B followed by C and the lowest in Q in the stem of *M. malabathricum* L. whilst all samples in the root and stem were not statistically significant in terms of TAC.

Table 4.17: Effects of different treatments on total anthocyanin content (TAC) in various parts of *M. malabathricum* L.

	TAC (mg g^{-1} DW)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	0.10 ± 0.04^{b}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
PK Biochar (B)	0.27 ± 0.04^{a}	0.06 ± 0.02^{a}	0.06 ± 0.03^{a}
FW Compost(C)	0.36 ± 0.03^{a}	0.03 ± 0.01^{a}	0.09 ± 0.02^{a}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; TAC: total anthocyanin content; DW: dry weight.

4.4.4.3 Total Phenolic Content (TPC)

The total phenolic content (TPC) in the methanolic extracts of the various parts of *M. malabathricum* L. was measured and expressed as mg gallic acid (GAE) per g dry extract. The TPC in the leaf extracts (Table 4.18) was observed to be similar among the three treatments with no significant difference observed among the treatments. The TPC of the stem extracts was observed to be significantly higher for treatment C (4930.96 ± 16.03 mg GAE g^{-1} DE) compared to B (2267.81 ± 58.94 mg GAE g^{-1} DE) and Q (2174.52 ± 27.79 mg GAE g^{-1} DE). A significantly higher TPC value was also found in the root methanolic extract of treatment C (5396.671 ± 8.20 mg GAE g^{-1} DE) and B (5419.29 ± 36.12 mg GAE g^{-1} DE) compared to Q.

Table 4.18: Effects of different treatments on total phenolic content (TPC) in various parts of *M. malabathricum* L. methanolic extracts.

	TPC (mg GAE g^{-1} DE)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	9505.16 ± 182.06^{a}	2174.52 ± 27.79^{b}	3607.00 ± 9.40^{b}
PK Biochar (B)	9933.32 ± 30.22^{a}	2267.81 ± 58.94^{b}	5419.29 ± 36.12^{a}
FW Compost(C)	9857.33 ± 49.17^{a}	4930.96 ± 16.03^{a}	5396.67 ± 8.20^{a}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; TPC: total phenolic content; GAE: gallic acid equivalent; DE: dry extract.

4.4.4.4 Total Flavonoid Content (TFC)

The total flavonoid contents (TFC) were measured and expressed as mg quercetin (QE) per g dry extract. A similar trend was observed between TFC and TPC values. The TFC in the leaf extracts (Table 4.19) was found to be significantly different for both treatment B and C compared to the control. In the stem extracts, a significantly higher TFC value was obtained in treatment C (209.98 ± 0.57 mg QE g^{-1} DE) compared to B (183.57 ± 0.92 mg QE g^{-1} DE) and Q (183.35 ± 1.26 mg QE g^{-1} DE). Meanwhile, a significantly higher TFC value in root methanolic extract was found for treatment B (643.27 ± 5.95 mg QE g^{-1} DE) followed by C (232.94 ± 2.51 mg QE g^{-1} DE) and Q (175.84 ± 4.40 mg QE g^{-1} DE).

Table 4.19: Effects of different treatments on total flavonoid content (TFC) in various parts of *M. malabathricum* L. methanolic extracts.

	TFC (mg QE g^{-1} DE)		
Treatments	**Leaves**	**Stem**	**Roots**
Control (Q)	1088.22 ± 31.54^{b}	183.35 ± 1.26^{b}	175.84 ± 4.40^{c}
PK Biochar (B)	1524.80 ± 38.13^{a}	183.57 ± 0.92^{b}	643.27 ± 5.95^{a}
FW Compost(C)	1464.90 ± 16.03^{a}	209.98 ± 0.57^{a}	232.94 ± 2.51^{b}

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; TFC: total flavonoid content; QE: quercetin equivalent; DE: dry extract.

4.4.4.5 Individual Flavonoids

The methanolic extract was analyzed through HPLC to determine the identity of the individual flavonoids present in the samples. The extracts were screened for rutin, quercetin, flavone, kaempferol and myricetin. The results indicated that rutin was present in each of the three treatments (Table 4.20). The results showed that the mean value of rutin accumulated greater in PK biochar (treatment B) with 54.15 ± 4.75 g^{-1} DW followed by FW compost (47.61 ± 6.07 g^{-1} DW) and control (41.57 ± 8.74 g^{-1} DW). Additionally,

the HPLC analysis also revealed the presence of other unidentified flavonoids in the stem and root extracts (Figure 4.9 and Figure 4.10). Stem extract for all treatments was found to contain unknown flavonoids with a comparable retention time (t_R). However, root extracts from treatment C had seven more unknown flavonoids separated at various t_R, whereas treatments B and Q contained four and two unknown flavonoids, respectively.

Table 4.20: Distribution and content of individual flavonoids present in *M. malabathricum* L. methanolic extracts.

	Individual flavonoids in leaves (mg g^{-1} DW)					
Treatments	**Rutin**	**Quercetin**	**Flavone**	**Kaempferol**	**Myricetin**	**Unknown peak (t_R in min)**
Control (Q)	41.574 ± 8.740[a]	ND	ND	ND	ND	-
PK Biochar (B)	54.148 ± 4.748[a]	ND	ND	ND	ND	-
FW Compost(C)	47.613 ± 6.074[a]	ND	ND	ND	ND	-
	Individual flavonoids in stem (mg g^{-1} DW)					
Control (Q)	ND	ND	ND	ND	ND	U1:3.59
PK Biochar (B)	ND	ND	ND	ND	ND	U1: 3.58
FW Compost(C)	ND	ND	ND	ND	ND	U1: 3.59
	Individual flavonoids in roots (mg g^{-1} DW)					
Control (Q)	ND	ND	ND	ND	ND	U1: 3.09 U2: 3.59
PK Biochar (B)	ND	0.095 ± 0.000[a]	0.003 ± 0.002	ND	ND	U1: 3.18 U2: 3.61 U3: 4.59 U4: 5.32
FW Compost(C)	ND	ND	0.015	ND	ND	U1: 3.06 U2: 3.17 U3: 3.58 U4: 4.14 U5: 4.30 U6: 4.58 U7: 5.30

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; DW: dry weight; ND: not detected; U: unknown.

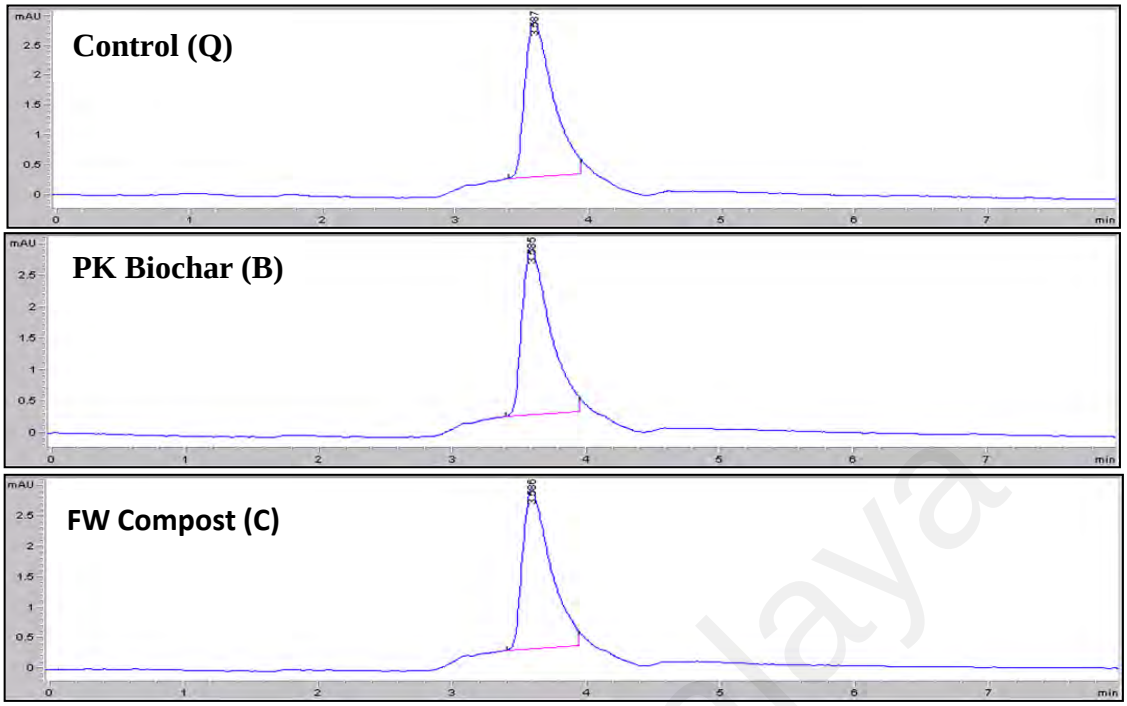


Figure 4.9: Flavonoids HPLC chromatograms showing the presence of unknown flavonoids in stem extracts of *M. malabathricum* L.

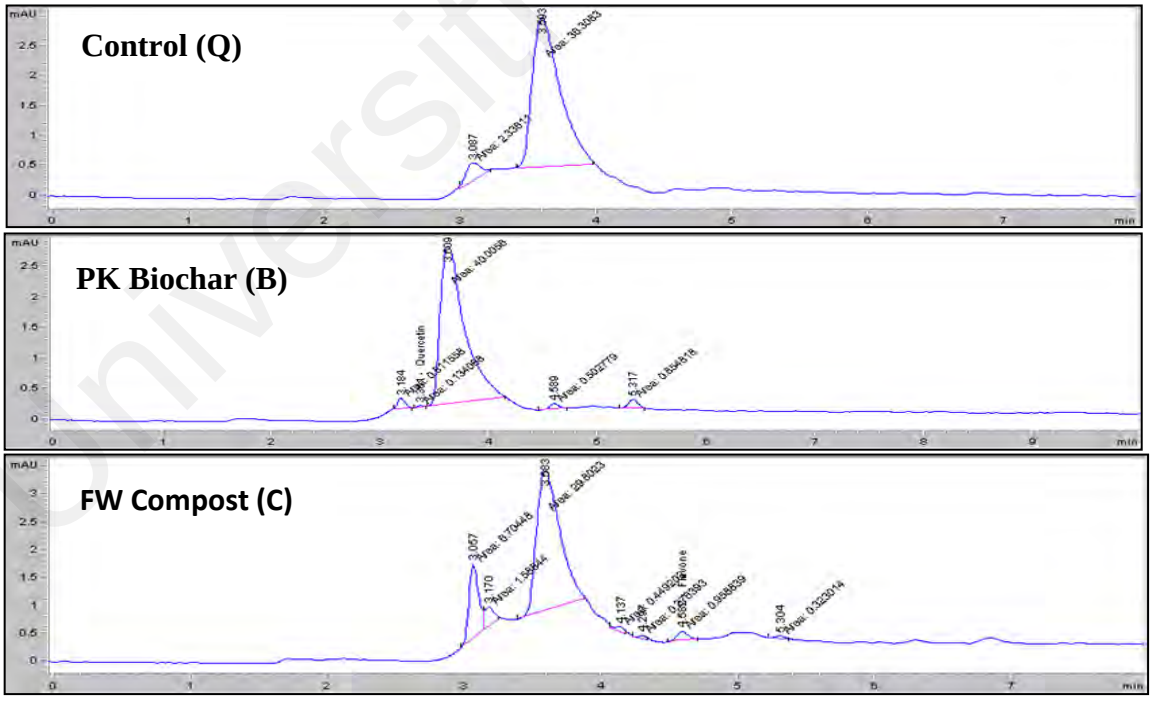


Figure 4.10: Flavonoids HPLC chromatograms showing the presence of unknown flavonoids in root extracts of *M. malabathricum* L.

4.4.4.6 Metabolite Profiling and Analysis

Overall, a total of 231 metabolites were annotated in both leaves and root extract with 129 and 102 potential metabolites in the leaves and root extract, respectively (Table 4.21).

Table 4.21: Summary of LCMS analysis

Number of potential metabolites		
Treatments	**Leaves**	**Roots**
Control (Q)	57	40
PK Biochar (B)	26	31
FW Compost(C)	46	31
TOTAL	129	102

a) List of Compounds in the Leaves Extract of *M. malabathricum* L. Subjected to Different Treatments

The major chemical compound constituents present in the leaves extracts of *M. malabathricum* L. grown on different treatments were listed in Table 4.22 – 4.24 while Figure 4.11- Figure 4.13 shows the total compound chromatogram (TCC) of the samples. The extracts were found to contain several significant chemical compounds, of which, many were found to be present in all treatments (Figure 4.17). In addition, 33 and 43 unidentified compounds were detected in the leaf and root samples, respectively.

Table 4.22: List of compounds detected in the leaves extract of *M. malabathricum* L. grown without soil amendment (Q)

No.	RT (min)	Base Peak (m/z)	Potential Compound	MF
1	0.632	219.0265	L-Galactose	$C_6H_{12}O_6$
2	0.646	385.097	-	$C_{13}H_{20}O_{13}$
3	1.27	282.1543	Ilicifolinoside A	$C_{11}H_{20}O_7$

Table 4.22, Continued

No.	RT (min)	Base Peak (m/z)	Potential Compound	MF
4	3.045 6.426	802.1093 802.1099	Pedunculagin	$C_{34}H_{24}O_{22}$
5	4.437 7.254	163.0388 163.0387	3-Hydroxycoumarin	$C_9H_6O_3$
6	4.444 7.254	355.1023 355.1028	Scopolin	$C_{16}H_{18}O_9$
7	6.783 8.353	339.1079 339.1072	4-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$
8	6.87 7.736 8.787	579.1494 579.1493 579.1503	Apigenin 7-(2"-E-p-coumaroylglucoside	$C_{30}H_{26}O_{12}$
9	7.055	307.081	(+)-Gallocatechin	$C_{15}H_{14}O_7$
10	7.254	291.0862	Oritin-4beta-ol	$C_{15}H_{14}O_6$
11	7.563	449.1075	6-C-Galactosylisoscutellarein	$C_{21}H_{20}O_{11}$
12	7.61	635.0868	Punicacortein A	$C_{27}H_{22}O_{18}$
13	7.612	617.0765	-	$C_{27}H_{20}O_{17}$
14	7.78	227.1278	12-hydroxyjasmonic acid	$C_{12}H_{18}O_4$
15	7.975	291.0863	Epifisetinidol-4alpha-ol	$C_{15}H_{14}O_6$
16	8.071	434.2016	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
17	8.107	412.2173	cis-3-Hexenyl b-primeveroside	$C_{17}H_{30}O_{10}$
18	8.301	558.2546	Sambacin	$C_{26}H_{36}O_{12}$
19	8.302	331.1538	Gibberellin A105	$C_{19}H_{22}O_5$
20	8.327	954.1194	Casuarictin	$C_{41}H_{28}O_{26}$
21	8.549	401.159	Italipyrone	$C_{22}H_{24}O_7$
22	8.871	465.1026	8-Hydroxyluteolin 8-glucoside	$C_{21}H_{20}O_{12}$
23	8.908 9.736	524.2492 524.2498	Limonoate	$C_{26}H_{34}O_{10}$
24	9.297	303.0503	3,5,7,2',5'-Pentahydroxyflavone	$C_{15}H_{10}O_7$
25	9.48	679.5128	-	$C_{36}H_{66}N_6O_6$
26	9.868	453.1184	Cinchonain Ib	$C_{24}H_{20}O_9$
27	9.952	552.2467	-	$C_{29}H_{26}N_8O_3$
28	10.154	552.2493	-	$C_{33}H_{33}N_3O_5$
29	11.117	724.4109	-	$C_{32}H_5N_{11}O_4S_2$
30	11.658	690.3713	-	$C_{31}H_{49}N_{13}O_3$
31	11.659	637.3234	-	$C_{34}H_{44}N_4O_8$
32	11.659	695.3264	-	$C_{32}H_{42}N_{10}O_8$
33	11.755 11.934	741.1825 741.1821	Kaempferol 3-(2"-(Z)-p-coumaryl-6"-(E)-p-coumarylglucoside)	$C_{39}H_{32}O_{15}$
34	11.858	743.3823	-	$C_{33}H_{54}N_6O_{13}$
35	11.859	738.4264	-	$C_{32}H_{55}N_{11}O_9$
36	11.911 11.914	662.3752 667.3307	Capsianoside IV	$C_{32}H_{52}O_{13}$
37	12.033	637.3213	-	$C_{33}H_{48}O_{12}$
38	12.035	736.4114	-	$C_{36}H_{65}NO_{10}S_2$

Table 4.22, Continued

No.	RT (min)	Base Peak (m/z)	Potential Compound	MF
39	12.312	290.2686	16-hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$
40	12.312	688.3539	-	$C_{34}H_{49}N_5O_{10}$
41	12.541	688.3891	-	$C_{39}H_{53}N_5O_4S$
42	12.862	451.3205	-	$C_{30}H_{42}O_3$
43	12.886	387.1797	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$
44	13.341	506.3847	Arjunolic acid	$C_{30}H_{48}O_5$
45	14.04	763.4231	Torvoside C	$C_{39}H_{64}O_{13}$
46	15.139 15.297 15.611	635.3951 635.3951 635.3951	2alpha-Hydroxypyracrenic acid	$C_{39}H_{54}O_7$
47	15.433 15.788	665.4054 665.4038	3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$
48	15.554	437.3413	Demethylphylloquinone	$C_{30}H_{44}O_2$
49	15.555 16.151	455.3522 455.3522	Bryononic acid	$C_{30}H_{46}O_3$
50	15.792	907.468	Polypodoside A	$C_{45}H_{72}O_{17}$
51	16.86	523.3992		$C_{31}H_{54}O_6$
52	16.863	887.498	Tuberoside D	$C_{45}H_{74}O_{17}$
53	17.221 17.418	619.3998 619.4004	3-O-cis-Coumaroylmaslinic acid	$C_{39}H_{54}O_6$
54	17.377	411.326	γ-Tocotrienol	$C_{28}H_{42}O_2$
55	17.433	740.4585	Lycoperoside D	$C_{39}H_{65}NO_{12}$
56	18.027	717.4196		$C_{40}H_{60}O_{11}$
57	19.631	593.2766	Pheophorbide a	$C_{35}H_{36}N_4O_5$

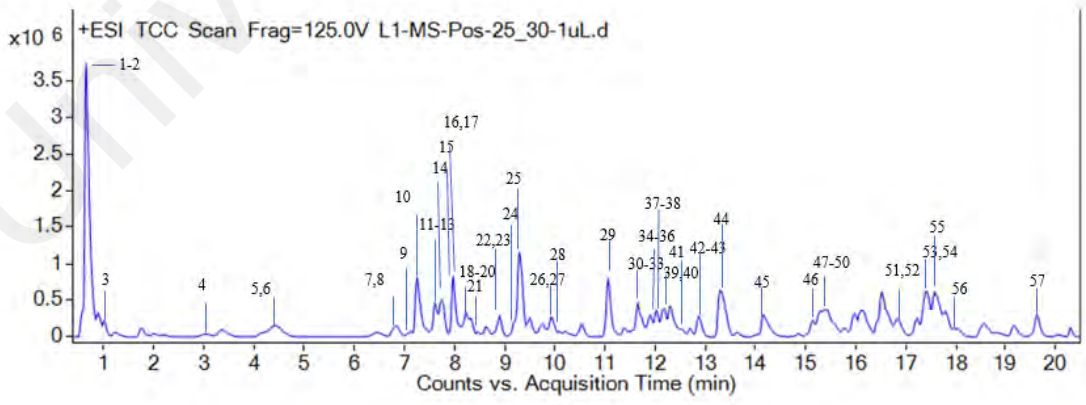


Figure 4.11: Total Compound Chromatogram (TCC) in leaves of control (Q). The peak numbers correspond to the annotated compounds as presented in Table 4.22

Table 4.23: List of compounds detected in the leaves extract of *M. malabathricum* L. grown with PK biochar (B)

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
1	0.932	224.0766	Methylisocitric acid	$C_7H_{10}O_7$
2	4.475 7.259	355.1024 355.1028	Scopolin	$C_{16}H_{18}O_9$
3	6.799	339.1076	4-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$
4	7.262	291.086	Oritin-4beta-ol	$C_{15}H_{14}O_6$
5	7.617	617.0755	-	$C_{24}H_{12}N_{10}O_{11}$
6	7.739	579.1502	Apigenin 7-(2"-E-p-coumaroylglucoside	$C_{30}H_{26}O_{12}$
7	7.98	291.086	Epifisetinidol-4alpha-ol	$C_{15}H_{14}O_6$
8	8.072	434.2025	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
9	8.87	465.1033	8-Hydroxyluteolin 8-glucoside	$C_{21}H_{20}O_{12}$
10	9.303	303.0499	3,5,7,2',5'-Pentahydroxyflavone	$C_{15}H_{10}O_7$
11	9.303	449.1087	6-Hydroxyluteolin 5-rhamnoside	$C_{21}H_{20}O_{11}$
12	9.481	679.5114	-	$C_{36}H_{66}N_6O_6$
13	10.154	552.2441	Undulatone	$C_{27}H_{34}O_{11}$
14	11.758	741.1814	Kaempferol 3-(2"-(Z)-p-coumaryl-6"-(E)-p-coumarylglucoside)	$C_{39}H_{32}O_{15}$
15	11.859	738.4268	-	$C_{33}H_{51}N_{15}O_5$
16	11.859	743.3819	-	$C_{33}H_{54}N_6O_{13}$
17	11.911 11.916	662.3748 667.3301	Capsianoside IV	$C_{32}H_{52}O_{13}$
18	12.249	318.3001	Phytosphingosine	$C_{18}H_{39}NO_3$
19	12.319	290.2686	16-hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$
20	12.89	387.1797	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$
21	13.341	506.3842	Arjunolic acid	$C_{30}H_{48}O_5$
22	15.046 16.862	909.4811 887.4972	Tuberoside D	$C_{45}H_{74}O_{17}$

Table 4.23, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
23	15.426 15.791	665.4062 665.4047	3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$
24	15.987 16.15	490.3882 473.3625	Lucidumol A	$C_{30}H_{48}O_4$
25	17.218 17.424	619.4005 619.4009	3-O-cis-Coumaroylmaslinic acid	$C_{39}H_{54}O_6$
26	17.437	740.459	Lycoperoside D	$C_{39}H_{65}NO_{12}$

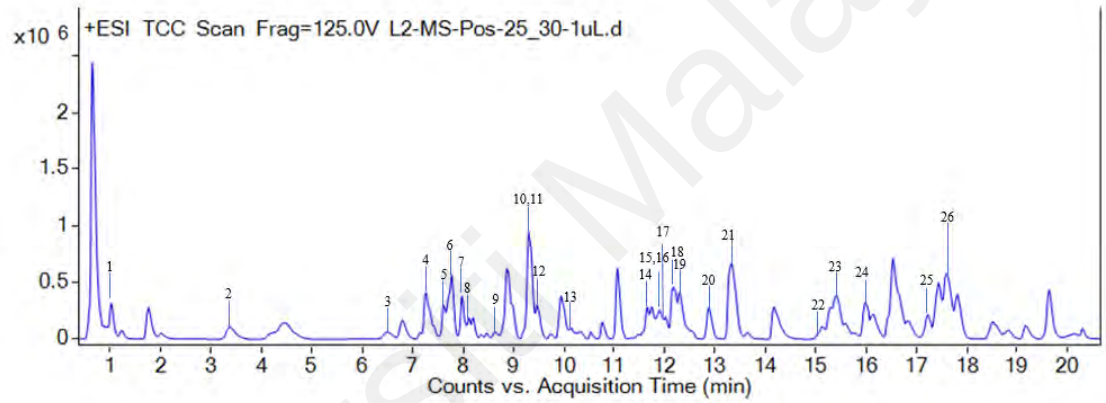


Figure 4.12: Total Compound Chromatogram (TCC) in leaves of PK biochar (B). The peak numbers correspond to the annotated compounds as presented in Table 4.23

Table 4.24: List of compounds detected in the leaves extract of *M. malabathricum* L. grown with FW compost (C)

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
1	0.631	219.0265	L-Galactose	$C_6H_{12}O_6$
2	0.64	249.0372	D-Glucoheptose	$C_7H_{14}O_7$
3	2.381	155.034	3,4-Dihydroxybenzoic acid	$C_7H_6O_4$
4	4.496 7.267	163.0387 163.0387	3-Hydroxycoumarin	$C_9 H_6 O_3$
5	4.498 7.263	355.1024 355.1026	Scopolin	$C_{16}H_{18}O_9$
6	6.804	339.1074	4-p-Coumaroylquinic acid	$C_{16}H_{18}O_8$

Table 4.24, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
7	6.884 7.742 8.79	579.1508 579.1507 579.1496	Apigenin 7-(2"-E-p-coumaroylglucoside	$C_{30}H_{26}O_{12}$
8	7.069	307.0808	Robinetinidol-4alpha-ol	$C_{15}H_{14}O_7$
9	7.099 10.339	595.1455 595.145	Kaempferol 3-(2"-(Z)-p-coumaroylglucoside)	$C_{30}H_{26}O_{13}$
10	7.267	291.086	Oritin-4beta-ol	$C_{15}H_{14}O_6$
11	7.985	291.087	Epifisetinidol-4alpha-ol	$C_{15}H_{14}O_6$
12	8.285	305.0647	Alphitonin	$C_{15}H_{12}O_7$
13	8.309	558.2549	Sambacin	$C_{26}H_{36}O_{12}$
14	8.552	401.1592	Italipyrone	$C_{22}H_{24}O_7$
15	8.718	611.1603	Robinetin 3-rutinoside	$C_{27}H_{30}O_{16}$
16	8.868	465.1035	8-Hydroxyluteolin 8-glucoside	$C_{21}H_{20}O_{12}$
17	8.914 9.726	524.2491 524.2481	Limonoate	$C_{26}H_{34}O_{10}$
18	9.126	625.1758	Tricetin 7-methyl ether 3'-glucoside-5'-rhamnoside	$C_{28}H_{32}O_{16}$
19	9.299	449.1088	6-Hydroxyluteolin 5-rhamnoside	$C_{21}H_{20}O_{11}$
20	9.299	303.0496	3,5,7,2',5'-Pentahydroxyflavone	$C_{15}H_{10}O_7$
21	9.478	679.5113	-	$C_{36}H_{66}N_6O_6$
22	9.953 10.147	552.2435 552.244	Undulatone	$C_{27}H_{34}O_{11}$
23	11.657	690.3704	-	$C_{33}H_{55}NO_{14}$
24	11.658	695.3262	-	$C_{32}H_{42}N_{10}O_8$
25	11.757	741.1819	Kaempferol 3-(2"-(Z)-p-coumaryl-6"-(E)-p-coumarylglucoside)	$C_{39}H_{32}O_{15}$
26	11.857	738.4276	-	$C_{33}H_{51}N_{15}O_5$
27	11.857	743.3824	-	$C_{33}H_{54}N_6O_{13}$
28	11.911 11.914	662.3746 667.3306	Capsianoside IV	$C_{32}H_{52}O_{13}$
29	12.538	693.3466	Scillaren A	$C_{36}H_{52}O_{13}$
30	12.889	387.1793	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$

Table 4.24, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
31	13.124 13.344	506.3836 506.384	Arjunolic acid	$C_{30}H_{48}O_5$
32	13.145	679.3308	-	$C_{32}H_{42}N_{10}O_7$
33	13.146	674.3742	-	$C_{33}H_{55}NO_{13}$
34	14.863	633.3785	Geissospermine	$C_{40}H_{48}N_4O_3$
35	14.952	609.3792	2-O-Protocatechuoylalphitolic acid	$C_{37}H_{52}O_7$
36	14.98	663.3887	-	$C_{40}H_{54}O_8$
37	15.139 15.292 15.613	635.3955 635.3959 635.3944	2alpha-Hydroxypyracrenic acid	$C_{39}H_{54}O_7$
38	15.435 15.79	665.4055 665.4051	3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$
39	16.856	523.3985	-	$C_{28}H_{46}N_{10}$
40	17.046	455.3521	Bryononic acid	$C_{30}H_{46}O_3$
41	17.047	437.3407	Manglupenone	$C_{30}H_{44}O_2$
42	17.208	619.3999	3-O-cis-Coumaroylmaslinic acid	$C_{39}H_{54}O_6$
43	17.378	437.3414	Demethylphylloquinone	$C_{30}H_{44}O_2$
44	17.79	275.2563	-	$C_{12}H_{30}N_{6O}$
45	18.034	717.4196	-	$C_{40}H_{60}O_{11}$
46	18.588	935.7133	Betulinic Acid	$C_{30}H_{48}O_3$

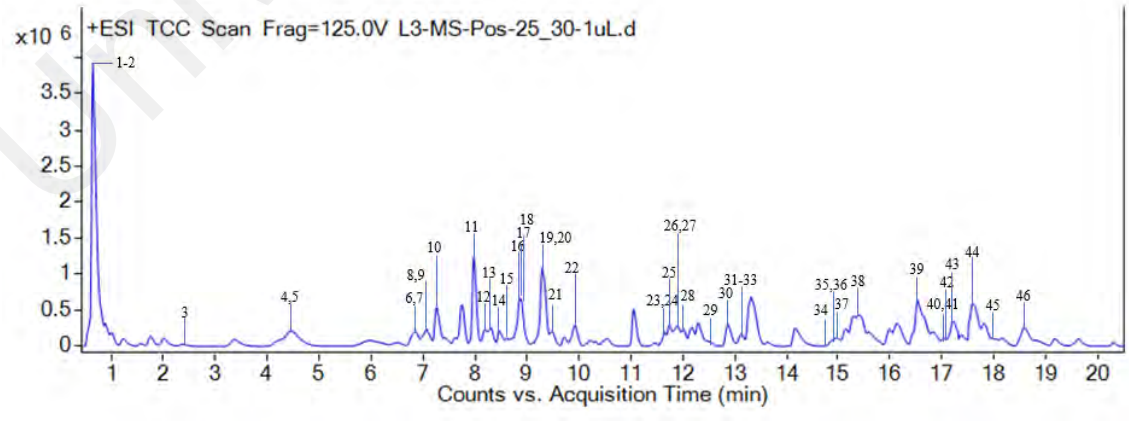


Figure 4.13: Total Compound Chromatogram (TCC) in leaves of FW compost (C). The peak numbers correspond to the annotated compounds as presented in Table 4.24

c) List of Compounds in the Root Extract of *M. malabathricum* L. Subjected to Different Treatments

The main chemical compound constituents present in the root extracts of *M. malabathricum* L. grown on different conditions are listed in Table 4.25 – Table 4.27, while the total compound chromatogram (TCC) of the samples is presented in Figure 4.14 – Figure 4.16.

Table 4.25: List of compounds detected in the root extract of *M. malabathricum* L. grown without soil amendment (Q)

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
1	6.853	398.2021	Prenyl apiosyl-(1->6)-glucoside	$C_{16}H_{28}O_{10}$
2	6.858	403.1576	3-Methyl-3-butenyl apiosyl-(1->6)-glucoside	$C_{16}H_{28}O_{10}$
3	7.348	420.1865	Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	$C_{18}H_{26}O_{10}$
4	7.353	425.1415	Benzyl beta-primeveroside	$C_{18}H_{26}O_{10}$
5	7.741	579.1497	Apigenin 7-(3"-p-coumaroylglucoside)	$C_{30}H_{26}O_{12}$
6	8.073	434.2018	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
7	8.345	420.2219	-	$C_{16}H_{25}N_{11}O_3$
8	8.493	564.3574	-	$C_{21}H_{42}N_{10}O_7$
9	8.632	608.3848	-	$C_{26}H_{54}O_{14}$
10	8.724	510.0884	-	$C_{21}H_{16}O_{14}$
11	8.755	652.411	-	$C_{28}H_{58}O_{15}$
12	8.803 8.807	482.2596 487.2149	Linalool oxide D 3-[apiosyl-(1->6)-glucoside]	$C_{21}H_{36}O_{11}$
13	8.873	348.7229	-	$C_{30}H_{62}O_{16}$
14	8.981	370.7352	-	$C_{30}H_{57}N_{15}O_7$
15	8.981	362.2218	-	$C_{15}H_{31}N_5O_3S$
16	9.478	679.5108	-	$C_{36}H_{66}N_6O_6$
17	9.521 9.727	435.1768 452.2026	-	$C_{21}H_{26}N_2O_8$
18	9.723	524.2499	Limonoate	$C_{26}H_{34}O_{10}$
19	9.803	464.2479	-	$C_{26}H_{33}N_5OS$
20	10.378	477.2224	-	$C_{24}H_{32}N_2O_8$
21	11.067	668.4367	Saponin H	$C_{36}H_{58}O_{10}$
22	12.246	318.2998	Phytosphingosine	$C_{18}H_{39}NO_3$
23	12.313	290.2686	16-hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$
24	12.398 12.864 12.87 14.407	469.3311 504.3681 509.3237 487.3417	Quillaic acid	$C_{30}H_{46}O_5$
25	12.406	522.379	Tomentosic acid	$C_{30}H_{48}O_6$

Table 4.25, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
26	12.888	387.1798	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$
27	13.331	506.3841	Arjunolic acid	$C_{30}H_{48}O_5$
28	13.462	393.2858	-	$C_{21}H_{33}N_3O_3$
29	14.123	625.3738	-	$C_{37}H_{52}O_8$
30	14.5	607.3638	-	$C_{37}H_{50}O_7$
31	14.561	681.3983	-	$C_{40}H_{56}O_9$
32	14.966	609.3801	2-O-Protocatechuoylalphitolic acid	$C_{37}H_{52}O_7$
33	15.299	635.3946	2alpha-Hydroxypyracrenic acid	$C_{39}H_{54}O_7$
34	15.444	665.4049	3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$
35	15.928	594.1596	Proanthocyanidin A1	$C_{30}H_{24}O_{12}$
36	16.255	631.4006	-	$C_{41}H_{50}N_4O_2$
37	17.425	619.4	3-O-cis-Coumaroylmaslinic acid	$C_{39}H_{54}O_6$
38	18.186	457.3683	Betulinic Acid	$C_{30}H_{48}O_3$
39	18.22	736.5334	-	$C_{46}H_{73}NO_4S$
40	19.505	577.3902	-	$C_{38}H_{48}N_4O$

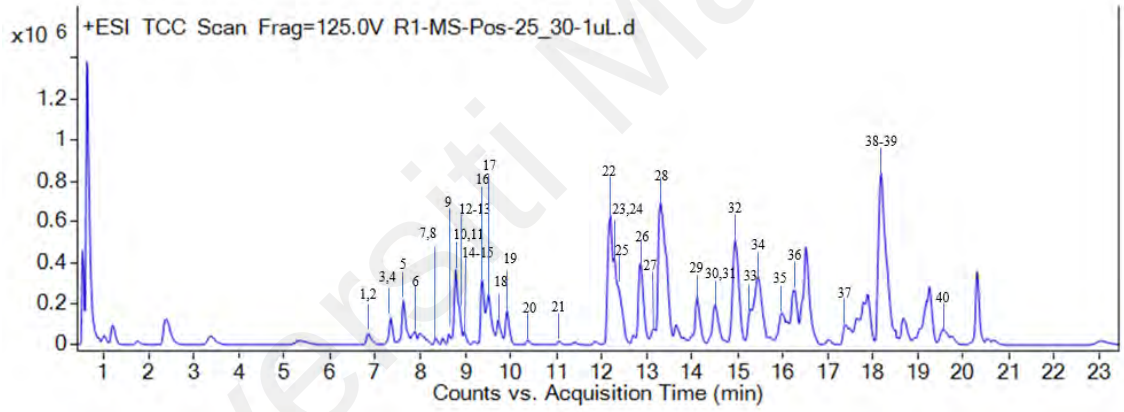


Figure 4.14: Total Compound Chromatogram (TCC) in root of control (Q). The peak numbers correspond to the annotated compounds as presented in Table 4.25

Table 4.26: List of compounds detected in the root extract of *M. malabathricum* L. grown PK biochar (B)

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
1	0.668	325.1125	3-Hydroxy-3-methyl-glutaric acid	$C_6H_{10}O_5$
2	7.348	420.1867	Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	$C_{18}H_{26}O_{10}$
3	7.499	441.1731	2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol 1-glucoside	$C_{19}H_{30}O_{10}$

Table 4.26, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
4	7.739	579.1497	Apigenin 7-(2"-E-p-coumaroylglucoside	$C_{30}H_{26}O_{12}$
5	7.799	313.1407	Tulipinolide	$C_{17}H_{22}O_4$
6	8.072	434.2024	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
7	8.472 8.814	482.2602 482.2596	Linalool oxide D 3-[apiosyl-(1->6)-glucoside]	$C_{21}H_{36}O_{11}$
8	8.631	608.3835	-	$C_{23}H_{46}N_{10}O_8$
9	8.728	317.0289	-	$C_{15}H_8O_8$
10	8.731	510.0886	-	$C_{21}H_{16}O_{14}$
11	9.48	679.5113	-	$C_{36}H_{66}N_6O_6$
12	9.524	435.1766	-	$C_{21}H_{26}N_2O_8$
13	9.721	345.1691	2-(4-Allyl-2-methoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1-propanol	$C_{20}H_{24}O_5$
14	12.315	290.2688	16-hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$
15	12.406	469.3311	Ganoderic acid DM	$C_{30}H_{44}O_4$
16	12.413	522.3791	Tomentosic acid	$C_{30}H_{48}O_6$
17	12.891	451.3205	-	$C_{30}H_{42}O_3$
18	12.873 12.875	995.6581 504.3681	Quillaic acid	$C_{30}H_{46}O_5$
19	12.891	387.1793	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$
20	13.181	520.3633	Esculentic acid (Phytolacca)	$C_{30}H_{46}O_6$
21	14.503	607.3639	-	$C_{38}H_{46}N_4O_3$
22	14.632	485.3257	Liquoric acid	$C_{30}H_{44}O_5$
23	14.997	663.3891	-	$C_{40}H_{54}O_8$
24	15.298	635.3952	2alpha-Hydroxypyracrenic acid	$C_{39}H_{54}O_7$
25	15.453	665.4065	3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$
26	15.544	437.3415	Demethylphylloquinone	$C_{30}H_{44}O_2$
27	15.73	490.3888	Lucidumol A	$C_{30}H_{48}O_4$
28	15.929	594.1608	Proanthocyanidin A1	$C_{30}H_{24}O_{12}$
29	17.426 17.591	619.3984 619.4001	3-O-cis-Coumaroylmaslinic acid	$C_{39}H_{54}O_6$
30	18.189	457.3672	Betulinic Acid	$C_{30}H_{48}O_3$
31	19.511	577.3903	-	$C_{38}H_{48}N_{4O}$

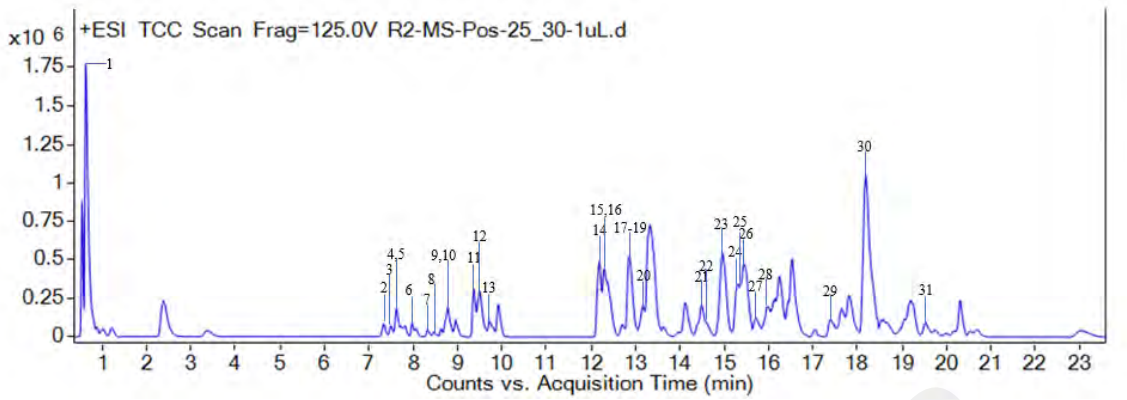


Figure 4.15: Total Compound Chromatogram (TCC) in root of PK biochar (B). The peak numbers correspond to the annotated compounds as presented in Table 4.26

Table 4.27: List of compounds detected in the root extract of *M. malabathricum* L. grown with FW compost (C)

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
1	0.65	543.1325	Panose	$C_{18}H_{32}O_{16}$
2	0.667	325.1131	(2R,3S)-2,3-Dimethylmalate	$C_6H_{10}O_5$
3	7.344	420.1861	Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	$C_{18}H_{26}O_{10}$
4	7.494	257.1379	2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol	$C_{13}H_{20}O_5$
5	7.494	436.2175	2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol 1-glucoside	$C_{19}H_{30}O_{10}$
6	7.582	406.2067	7-Epi-12-hydroxyjasmonic acid glucoside	$C_{18}H_{28}O_9$
7	7.624	307.0839	-	$C_{12}H_{18}O_7S$
8	7.852	471.183	-	$C_{20}H_{32}O_{11}$
9	8.068	434.2016	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$
10	8.493	564.3588	-	$C_{24}H_{50}O_{13}$
11	8.632	608.3855	-	$C_{22}H_{50}N_6O_{12}$
12	8.725	317.029	-	$C_{15}H_8O_8$
13	8.729	510.088	-	$C_{21}H_{16}O_{14}$

Table 4.27, Continued

No.	RT (min)	Base Peak (m/z)	Potential compound	MF
14	8.76	652.4099	-	$C_{26}H_{46}N_{14}O_5$
15	9.48	679.5127	-	$C_{36}H_{66}N_6O_6$
16	9.52	435.177	-	$C_{21}H_{26}N_2O_8$
17	9.603	699.1347	-	$C_{36}H_{26}O_{15}$
18	9.727	452.2014	-	$C_{18}H_{18}N_{12}O_2$
19	10.26	301.0699	6-Methylluteolin	$C_{16}H_{12}O_6$
20	12.412	522.3785	Tomentosic acid	$C_{30}H_{48}O_6$
21	12.721	518.3469	Ganoderic acid beta	$C_{30}H_{44}O_6$
22	12.865 14.413	504.3683 487.3411	Quillaic acid	$C_{30}H_{46}O_5$
23	12.865	451.3207	-	$C_{30}H_{42}O_3$
24	12.888	387.1797	4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	$C_{22}H_{26}O_6$
25	13.116 13.339	506.3839 506.3838	Arjunolic acid	$C_{30}H_{48}O_5$
26	13.26 13.424	511.3385 511.3382	Camelliagenin B	$C_{30}H_{48}O_5$
27	14.119	625.3738	-	$C_{37}H_{52}O_8$
28	14.497	607.3622	-	$C_{37}H_{50}O_7$
29	16.522	149.0231	-	$C_8H_4O_3$
30	18.182	457.3669	Betulinic Acid	$C_{30}H_{48}O_3$
31	18.197 19.744	439.3571 439.3566	Ganoderol A	$C_{30}H_{46}O_2$

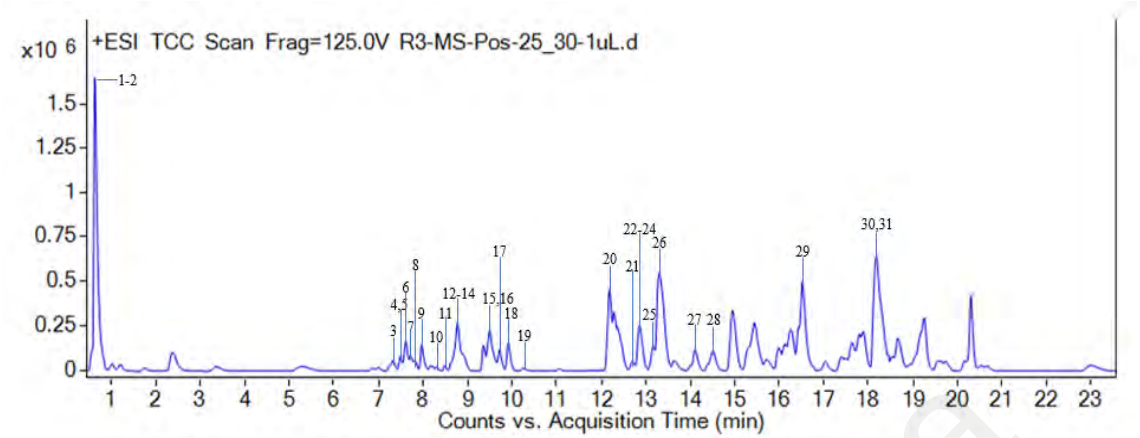


Figure 4.16: Total Compound Chromatogram (TCC) in root of FW compost (C). The peak numbers correspond to the annotated compounds as presented in Table 4.27

A comparison was made between the leaf extracts of B and C to see the effect of different soil amendments on metabolite profiles when used in comparison with control (Q). Of the total 129 aligned and deconvoluted features between treatments Q, B and C, Q recorded the highest number of metabolites (57) followed by C (46) and B (26), of which 15 features were shared among the three treatment groups (Figure 4.17). A total of 10 features were shared between Q and C followed by 4 features between Q and B and 3 features between B and C. To elucidate the difference between control and treatment application, a comparison was made between Q and B (Figure 4.18) and between Q and C (Figure 4.19). A total of 19 features were shared between Q and B, whereas 25 features were common between Q and C. A comparison between treatment B and C (Figure 4.20) revealed that treatment B possesses 8 distinct metabolites, while treatment C with 28 metabolites. Despite the differences, both treatments share an overlap of 18 metabolites.

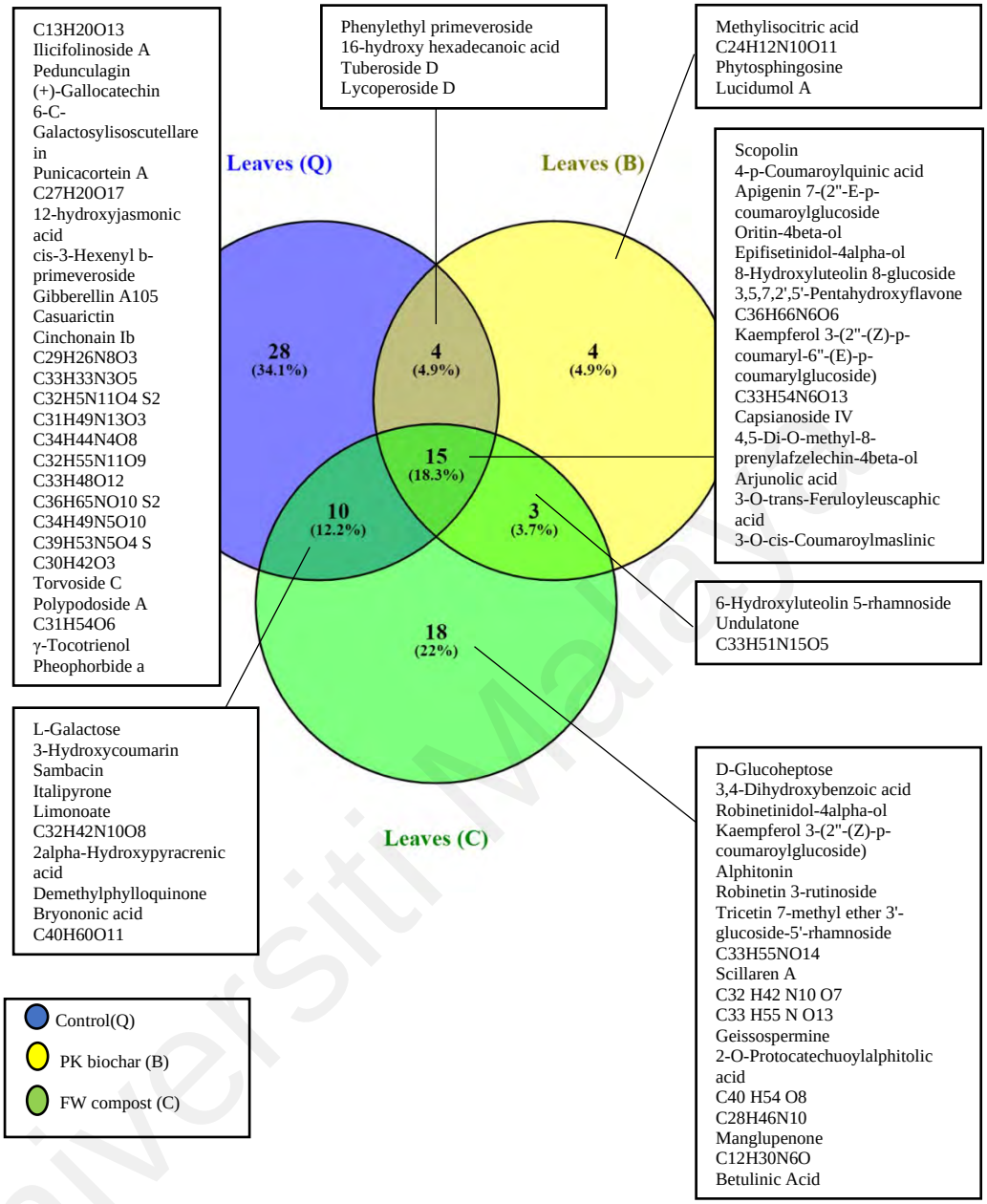


Figure 4.17: Venn diagrams showing the distribution of compounds in the leaves extract in treatment Q, B and C

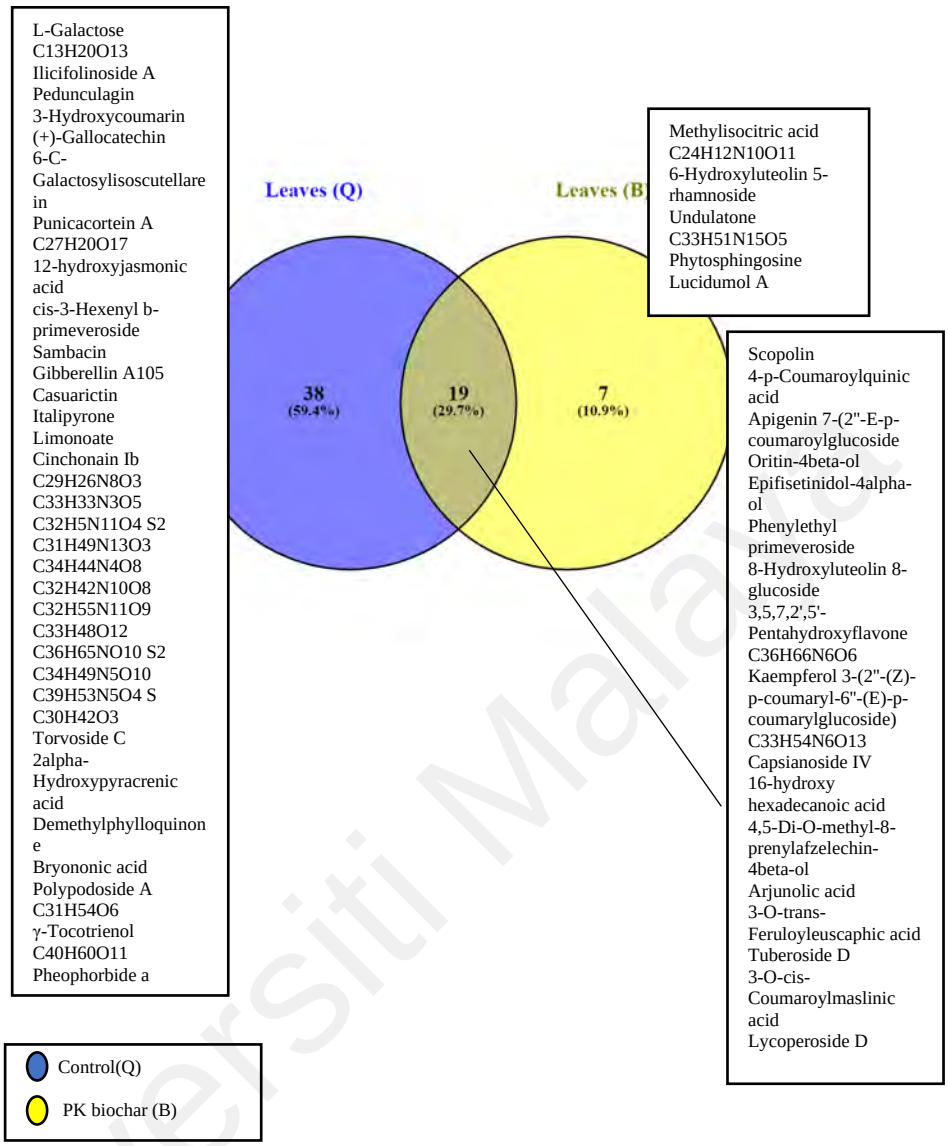


Figure 4.18: Venn diagrams showing the distribution of compounds in the leaves extract between treatment Q and B

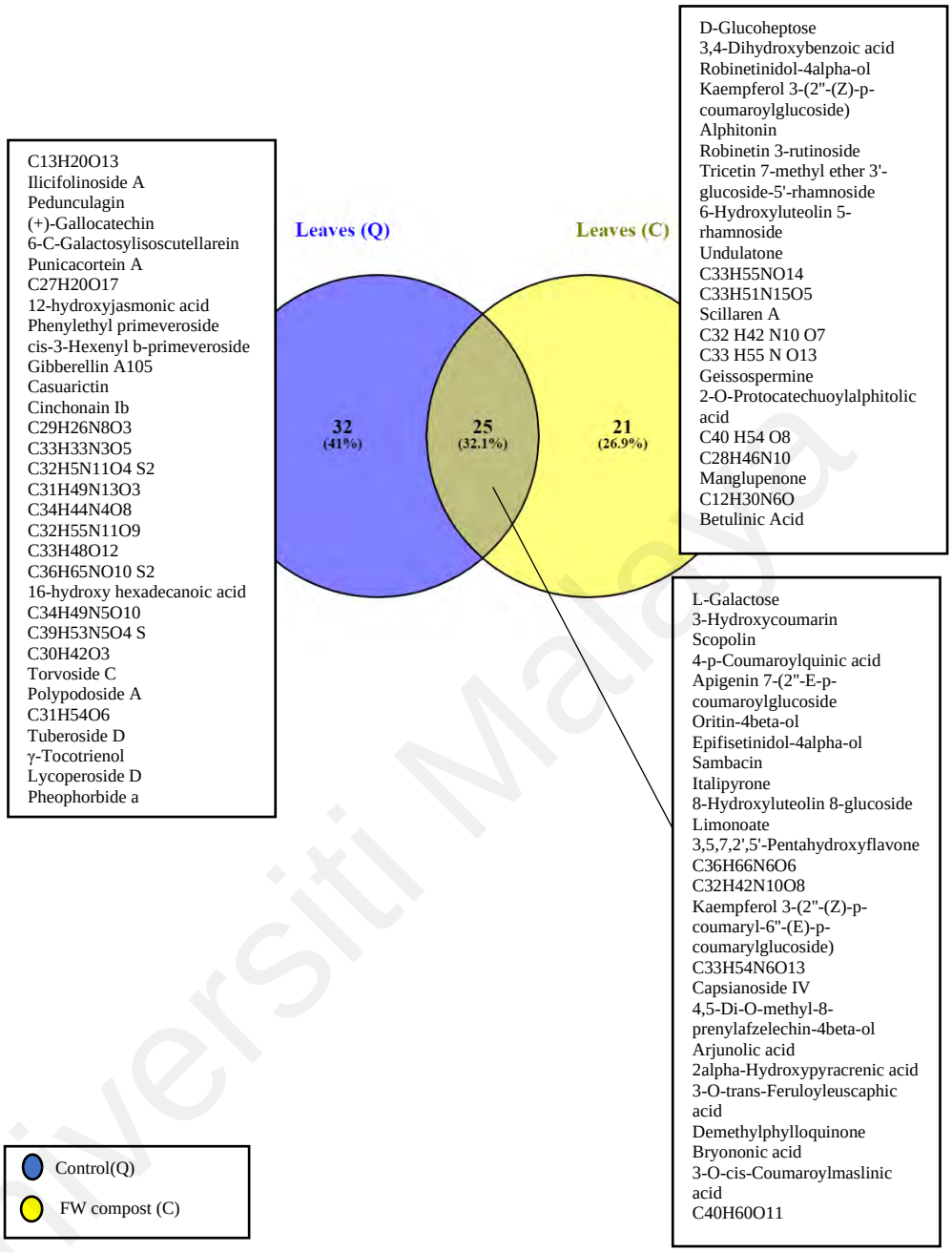


Figure 4.19: Venn diagrams showing the distribution of compounds in the leaves extract between treatment Q and C

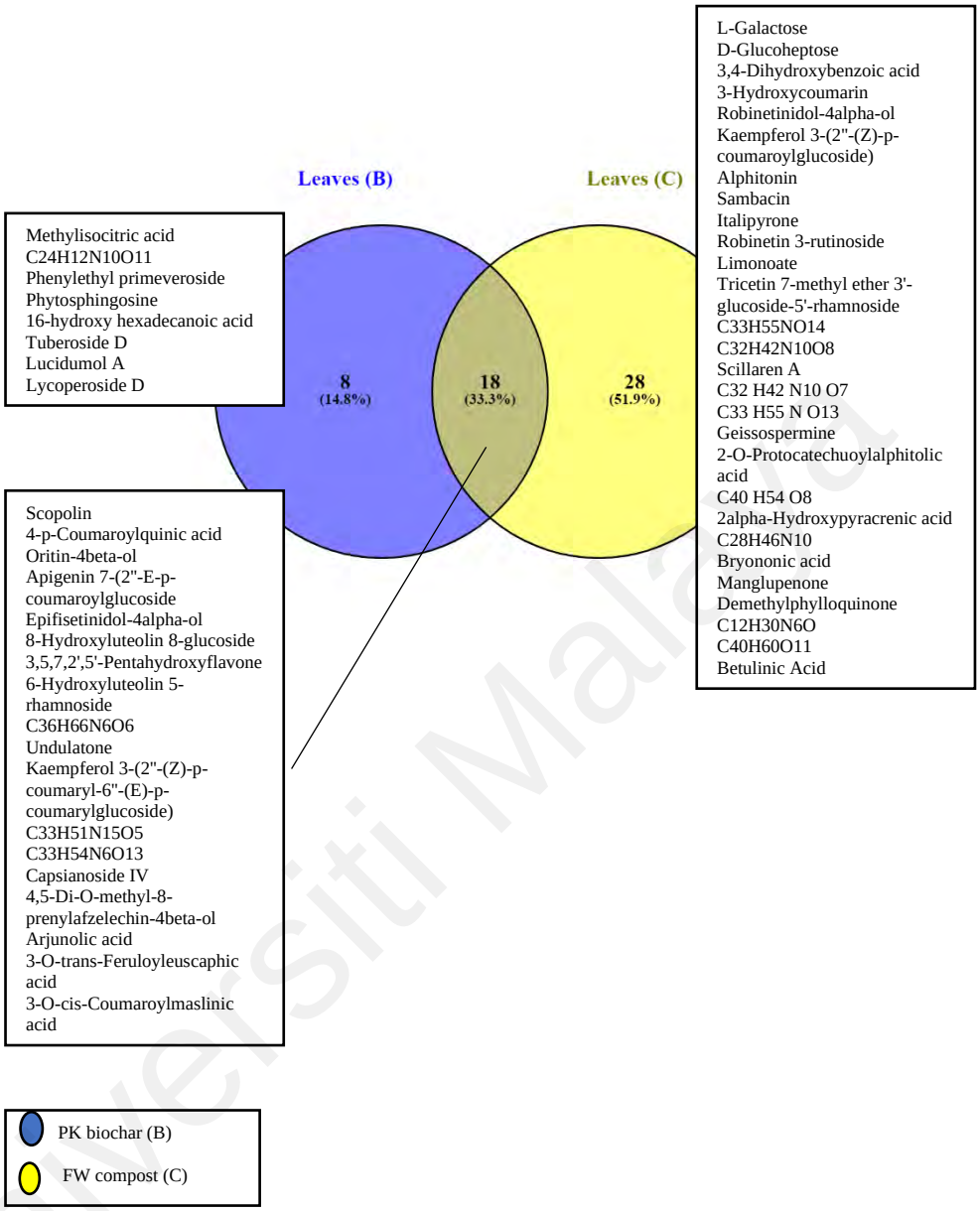


Figure 4.20: Venn diagrams showing the distribution of compounds in the leaves extract between treatment B and C

For root extract of treatment Q, B and C, a total of 102 aligned and deconvoluted features, a similar trend was observed with Q recording the highest number of metabolites (40) followed by C (31) and B (31), of which 9 features were shared among the three treatment groups (Figure 4.21). A total of 7 features were shared between Q and B, 3 features were shared between Q and C and 3 features were shared between B and C. To

elucidate the difference between control and treatment application in the root extract, a comparison was made between Q and B and between Q and C. A total of 16 features were shared between Q and B (Figure 4.22), whereas 12 features were common between Q and C (Figure 4.23). A comparative analysis was also conducted to investigate the effects of the soil amendment treatments between B and C. Treatment B exhibit 28 metabolites, whereas treatment C recorded 19 metabolites, with sharing features of 12 metabolites. Comparing treatment B and C (Figure 4.24), it was found that treatment B and treatment C contain 19 distinct metabolites, respectively, with both treatments have 12 metabolites in common.

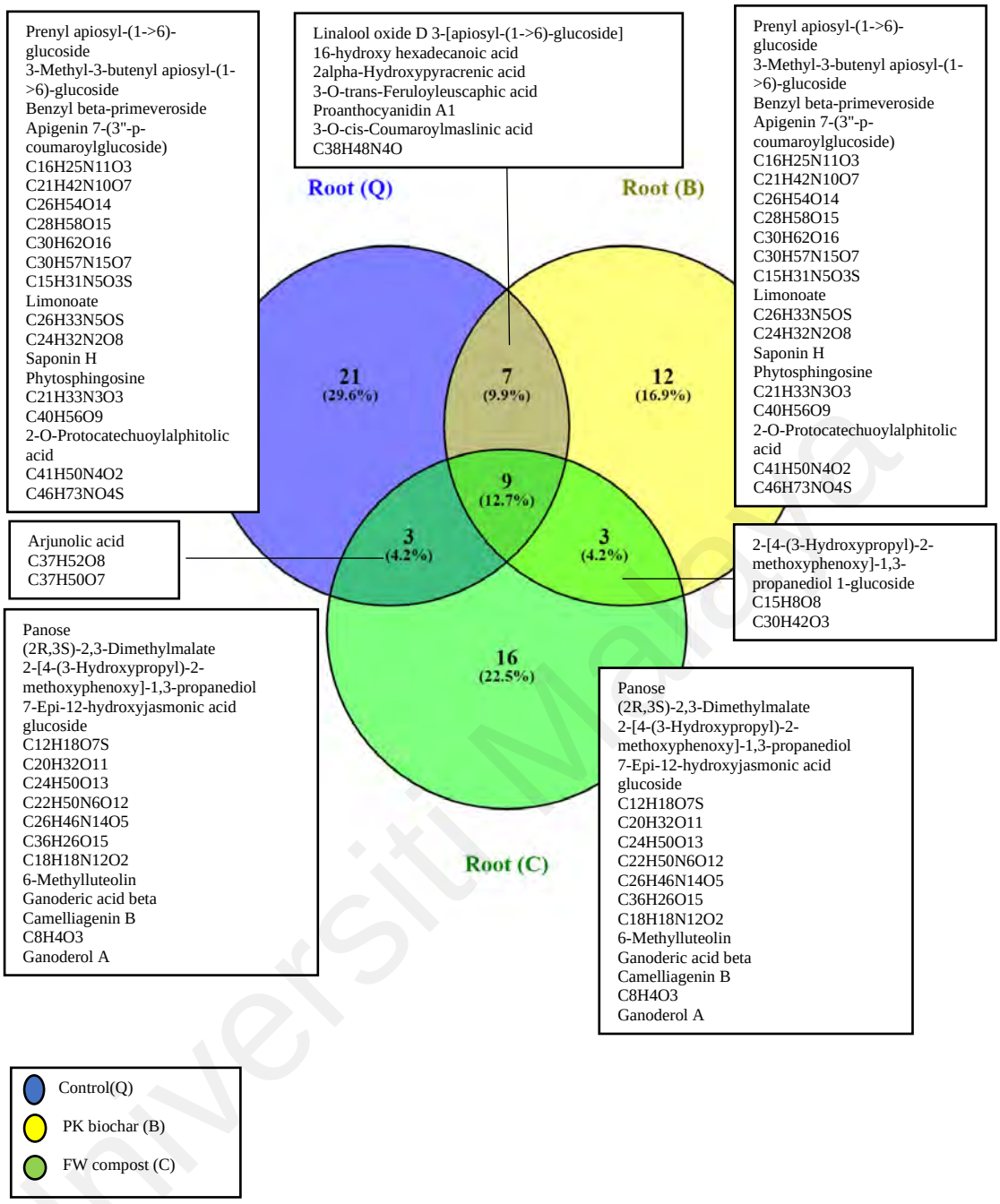


Figure 4.21: Venn diagrams showing the distribution of compounds in the root extract in treatment Q, B and C

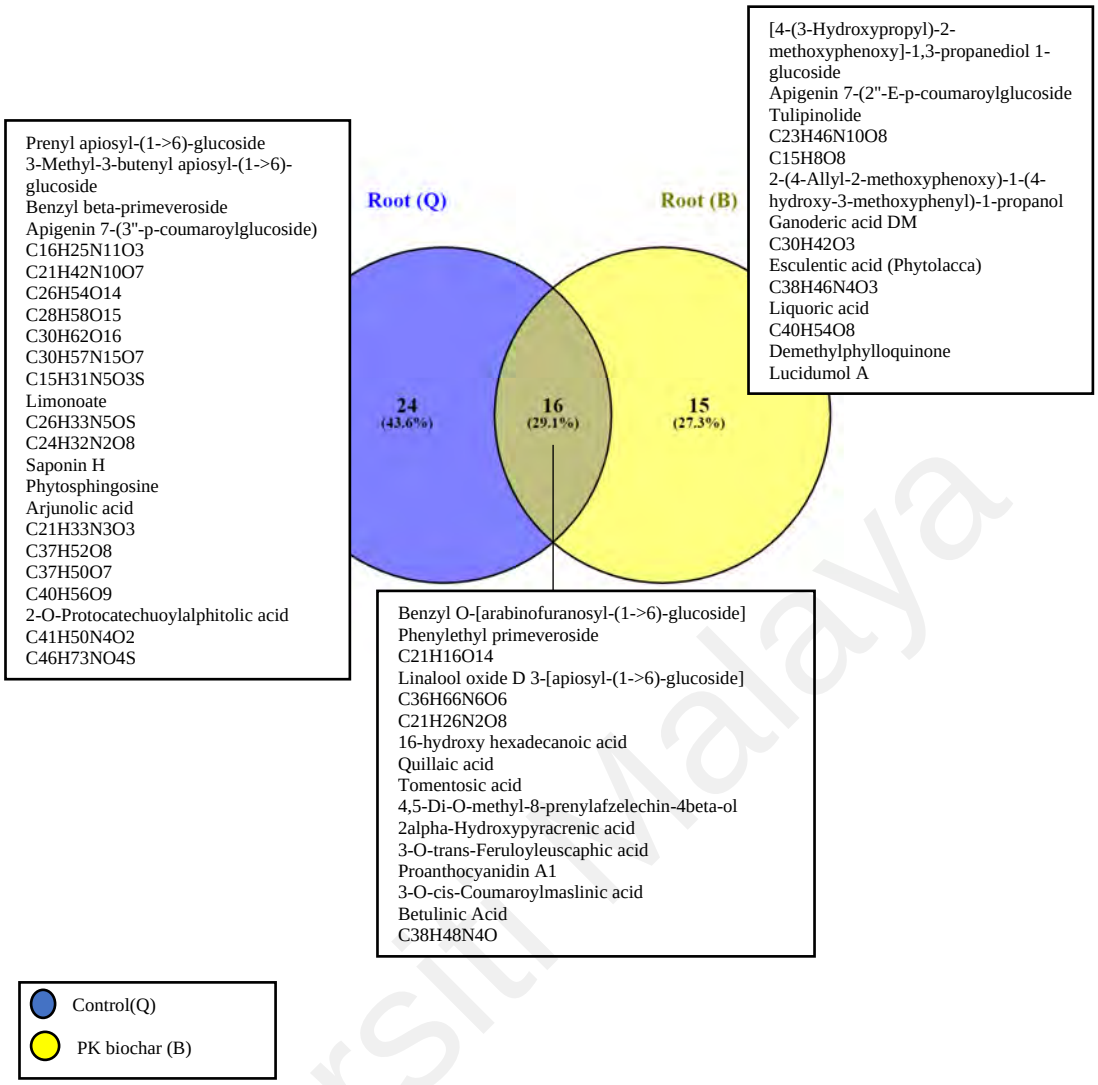


Figure 4.22: Venn diagrams showing the distribution of compounds in the root extract between treatment Q and B

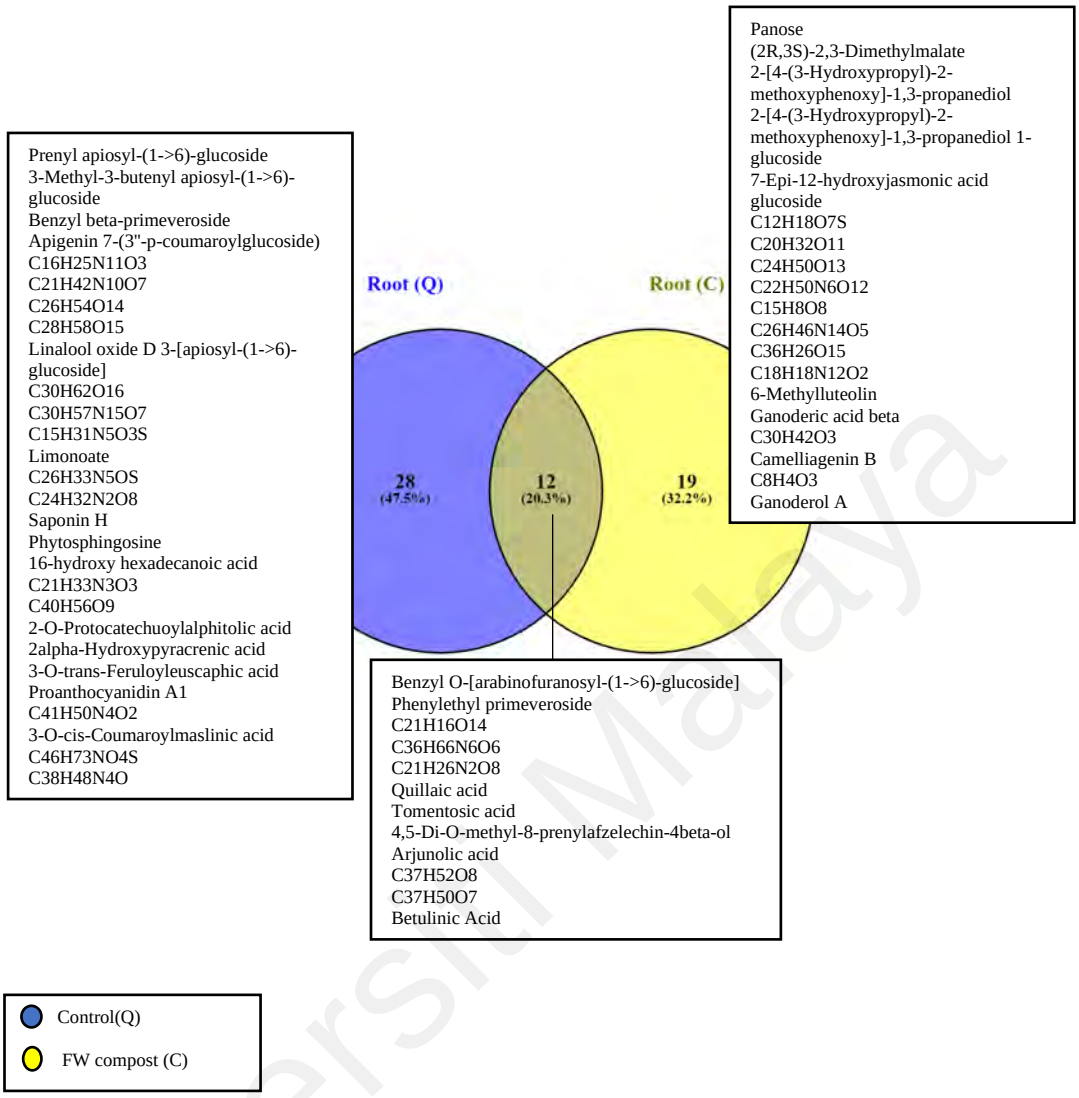


Figure 4.23: Venn diagrams showing the distribution of compounds in the root extract between treatment Q and C

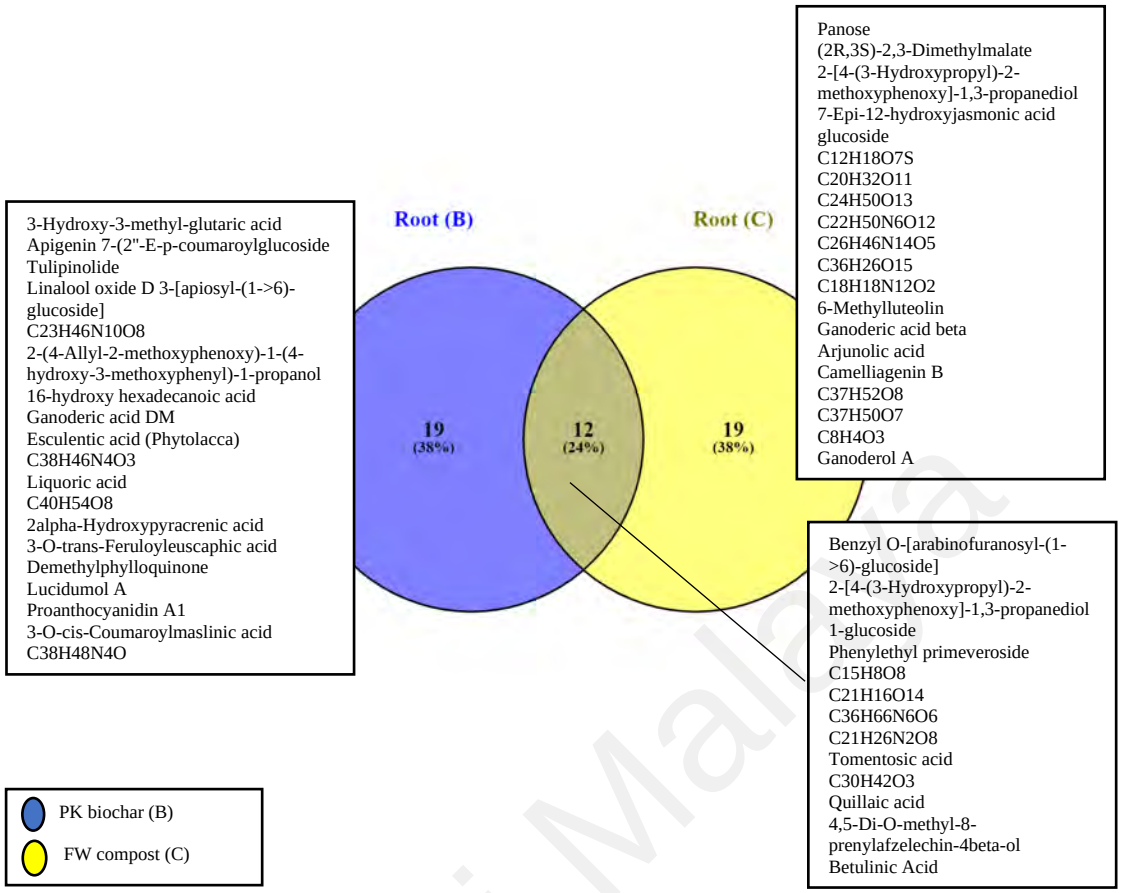


Figure 4.24: Venn diagrams showing the distribution of compounds in the root extract between treatment B and C

4.5 Effects of Different Soil Amendment Approaches on The Distribution of Auxin

The extract was screened for indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and indole-3-propanoic acid (IPA) (Table 4.28). Generally, the level of auxin IAA, IBA and IPA was lower than the limit of quantification (LOQ) and thus could not be detected. The results indicated that IBA was present in the leaf extract for treatment B (1.3 mg kg^{-1} DW). Meanwhile, the presence of IPA was observed in the root extract for the control treatment (10.9 mg kg^{-1} DW).

Table 4.28: Differential distribution of auxin

Treatments	IAA	IBA	IPA	Unknown peaks (t_R in min)
Auxin in leaves (mg kg^{-1} DW)				
Control (Q)	ND	ND	ND	1.821 2.066 5.906 13.439
PK Biochar (B)	ND	1.3	ND	2.118 5.611 8.033 13.583
FW Compost(C)	ND	ND	ND	2.093 9.038 13.543
Auxin in roots (mg kg^{-1} DW)				
Control (Q)	ND	ND	10.9	1.067 1.873 3.965 6.036 8.701 11.045 13.627
PK Biochar (B)	ND	ND	ND	4.091 6.046 8.770 9.079 11.053 13.601
FW Compost(C)	ND	ND	ND	4.064 5.979 8.565 11.087 13.167 13.535 14.500

The values (mean ± SE) followed by dissimilar letters in each column are significantly different at $p \leq 0.05$; DW: dry weight; ND: not detected below limit of quantification (LOQ=0.5)

CHAPTER 5: DISCUSSION

5.1 The Effect of Soil Amendments on the Growth and Nutrient Uptake of *M. malabathricum* L. in Tropical Acidic Soil Conditions

5.1.1 Plant Growth

The results of this study indicated that the tallest *M. malabathricum*'s L. plants were obtained when the plants were grown with PK biochar (treatment B). The greatest increment in the stem diameter was also recorded in treatment B. This result exhibits the potential of biochar in promoting *M. malabathricum* L. growth due to the beneficial nutrient contents retained and high water holding capacity of palm kernel shell biochar (Kong et al., 2019). The oxidation of biochar surfaces by biotic and abiotic processes and their further interactions in soil may increase cation exchange capacity significantly. Biochars' nutrient sorption capabilities may have significantly improved, which could lower NH_4^+-N leaching from soils (Cheng et al., 2006). Given the pH-raising effects of the amendment treatments, it was anticipated that the increase in plant height and stem diameter was attributable to the increased nutrient availability in the soil following organic amendment incorporation. In this study, a significantly higher N content was observed in treatment B compared to the control. The positive effect of nitrogen on rapid vegetative development can be attributed to the increase in plant height (Khan et al., 2008). The growth in the control plant is hindered as it suffers from a deficiency in N concentration (Souza et al., 2007). Similar findings were recorded in a previous study where oat plant height was increased with biochar treatment compared to compost (Schulz et al., 2013).

An increasing trend was observed though there were no significant differences in the

leaf area index (LAI) of *M. malabathricum* L. throughout the planting period in all treatments. The leaf area index of *M. malabathricum* L. increased with time throughout this study. The addition of PK biochar (treatment B) demonstrated the most prominent increase in LAI. This was likely caused by the application of biochar, which helped to improve the bioavailability of soil nutrients, as well as enhancement of soil fertility, which was proven by the higher leaf area index (Bolat et al., 2016; Qayyum et al., 2015). Furthermore, regular irrigation during the growing period helps to increase the coverage of the leaf area (Yuan et al., 2012).

Overall, the root length and root length density displayed a similar trend of response toward the treatments. Meanwhile, the average diameter and root volume also exhibited a similar style of treatment response. PK biochar (B) treatment displayed the highest root length and root length density, which was 20.25% higher than the control (Fig. 4.4a, b). The three combined amendments (BCL) had the lowest root average diameter and root volume after six months of observation (Fig. 4.4c, d). The results indicated that the macronutrients such as calcium contained in biochar helped to enhance the growth of the root (Gunarathne et al., 2017). This was parallel to the finding by Masto et al. (2013), where the increased concentrations of several nutrients in the plant tissues were noticed with the application of biochar. Furthermore, the acidic soil, which hinders root growth, can be mitigated through the application of biochar. The utilization of PK biochar likely disrupted these barriers by elevating soil pH and soil porosity, leading to a significant increase in root length (Singh et al., 2022). Moreover, biochar produced through the process of pyrolysis exhibited enhanced efficacy in promoting root elongation, attributed to its alkaline properties and larger surface area (Sohi et al., 2010; Yuan et al., 2011) . As a result, this facilitated deeper root penetration, allowing for a higher uptake of nutrients from the soil solution (Lehmann et al., 2015). Furthermore, Kumar et al. (2010) defined

that one of the most promising characteristics for better physiological performance is the high values of root length.

Amongst the treatments, the application of FW compost (treatment C) yielded the highest leaf biomass. The use of compost and biochar alone or together in combination with fertilizer improved the chlorophyll content and leaf biomass, which can also indicate increased availability of macro and micronutrients (Mankasingh et al., 2011). This leads to the production of vigorous plant growth and healthier plants, thus increasing vegetable biomass (Agegnehu et al., 2015). In addition, the use of biochar as an amendment provides an ideal environment where the high nanoporosity and low density of the biochar improve the aeration and compost structure (Zhang et al., 2014). Besides, the large porosity and surface area enable sufficient microbial growth, which increases the retention of plant nutrients through biochar application (Sanchez-Monedero et al., 2018). PK biochar (treatment B) exhibited the highest stem biomass, which could possibly be due to its ability to increase plant-available phosphorus in soil and hence increase plant growth (Gunarathne et al., 2017). Moreover, *M. malabathricum* L. grown with biochar and liming (BL) demonstrated the highest root biomass after six months of planting. The use of the liming materials improved the soil pH as well as decreased soil acidity, hydrogen and aluminium exchangeability (Onwuka et al., 2009). The current study found liming to have increased the root biomass due to soil pH increment, which was in accordance with the finding by Htwe et al. (2016).

5.1.2 Nutrient Uptake

In this study, the increase in macronutrient uptake (N, P and K) could be a result of the soil pH being treated by amendments used. A study by Huang et al. (2017) discovered that liming is able to significantly increase the N uptake by rice in the late growing season.

Similar to this study, lime treatment (L) significantly increased N uptake by *M. malabathricum* L. and this is comparable with PK biochar (B) and FW compost (C). This study demonstrated that lime, PK biochar and FW compost application in acidic soil were able to improve soil pH. The increase in soil pH affects soil fertility, and hence the nitrogen content through nutrient cycling by beneficial soil microorganisms through their chemical and biochemical activities (Turner, 2010). Additionally, soil supplemented with biochar is capable of retaining the nutrients contained and reducing leaching losses in the soil (Biederman et al., 2013; Laird et al., 2010). Biochar can have a liming effect that includes reducing the amount of P fixed by Al and Fe oxide (Yuan et al., 2011) and organic P was released into the atmosphere as a result of organic matter decomposition (Guppy et al., 2005). In addition, fertilization with organic compost made from household food waste improved nutrient uptake in leaf tissue (Ferreira et al., 2018).

With reference to Table 4.3, the soil pH at the final planting stage was significantly influenced by the soil amendments compared to the control. Hydrolysis occurs when the cation's charge or size ratio is sufficiently large to disrupt the H-O bonds, leading to hydrate ionization which produces hydrogen ions (Serrano, 2005). Moreover, the presence of Al promotes the development of most plants acclimated to low pH soils in tropical and temperate locations, which is believed to be related to the increased N, P and K uptake (Osaki et al., 1997). This explains the decrement of soil acidity up to 14% in *M malabathricum* L. in acidic soil conditions during the final planting stage. This result is in agreement with the previous study, which has shown that Al accumulator plants adapted to low soil pH (Osaki et al., 1997; Watanabe et al., 1998). Furthermore, the decrease in soil acidification occurred as a result of the soil's inherent Ca and Mg content being released. As the loss of basic cations by leaching is minimal, it is possible to increase soil pH through the accumulation of basic cations throughout the planting period

(Ch'ng et al., 2016).

Organic amendment treatments improved soil N by preventing NH_4^+ and NO_3^- from being lost (Paulin et al., 2008; Yao et al., 2012). FW compost and PK biochar's N content may have contributed to the rise in the total nitrogen in the soil. Soil N was significantly affected by the application of organic amendments (Table 4.4) and the findings indicated that both organic amendments increased soil nitrogen similarly. The highest percentage of soil N was recorded in the soil amended with FW compost, which was expected, given the fact that FW compost contained a substantially higher proportion of N compared to PK biochar. A study by Sierra et al. (2001) examined the effect of leaching on the depletion of nutrients, particularly nitrogen, from the soil. Evidently, simultaneous leaching of sodium out of the soil improves the circumstances for microbial activity, therefore, N was immobilized by moving to the microbial biomass triggered by organic matter decomposition.

Treatment with FW compost (C) showed the highest amount of available P in the soil (Table 4.4). Additionally, the high affinity of functional groups in the inputs of organic soil amendments may have inhibited P fixation by Al and Fe (Cheng et al., 2008). The presence of more soil-available P in the FW compost treatment than in the PK biochar treatment indicated that compost is a more efficient fertilizer in terms of supplying available P nutrients (Mkhabela et al., 2005). This may be associated with the increased microbial activity following the compost application and the P released during organic matter decomposition (Iyamuremye et al., 1996). This is followed by the treatment with PK biochar. Being more negatively charged, biochar readily binds to positively charged metal oxides such as Al_2O_3 and Fe_2O_3, a reaction that reduces the tendency of Al and Fe from reacting with soil-available P (Cheng et al., 2008)

Based on the organic amendments' nutrient composition, organic amendments might be expected to have a fertilizing effect on soil exchangeable bases, K, Ca and Mg. In this study, the observed increase in mineralization following soil amendment with FW compost could be attributed to the chemical composition of the FW compost used. This thus suggests that the chemical properties of the organic matter in the compost and the concentration of humic acids released into the soil influence the aggregate stability (Hernández et al., 2016; Piccolo et al., 1997; Whalen et al., 2003). The improvement in soil productivity and fertility associated with the exogenous addition of organic matter will endure in the soil so long as the added organic matter is protected from microbial attack (García-Ruiz et al., 2012; Hernández et al., 2016). Basic cations such as Ca^{2+}, Mg^{2+} and K^{+} in the form of oxides or carbonates can dissolve in water and produce OH^{-}, which in turn increases the soil pH (Berek et al., 2016; Smider et al., 2014). The carbonate content is responsible for the alkalinity of biochar (Hass et al., 2012; Mukome et al., 2013; Yuan et al., 2011) and was positively correlated with basic cation (Berek et al., 2018). This could be attributed to the release of basic cations from both FW compost and PK biochar. High K content in the PK biochar caused the reduced uptake of Ca in PK biochar (Table 4.2). Previous studies corroborated this finding (Kizito et al., 2019; Sigua et al., 2016) by reporting a negative relationship between Ca and K. Based on this statement, the high input of K from PK biochar could be attributed to the low uptake of Ca in PK biochar.

Concerning potentially hazardous trace elements, which is the most researched risk in soil modified with urban waste, the components found in greater amounts in this study were Fe > Cu > Zn > Mn > Cd > Al (Table 4.5). The results confirmed the hypothesis that the use of FW compost and PK biochar as soil amendments improved the micronutrient contents. Our results indicated that the concentrations of Cu and Zn

coincide with the concentration ranges for all Peninsular Malaysian topsoil (Zarcinas et al., 2004). However, Cd exceeded the maximum threshold level (Zarcinas et al., 2004). This result has also been reported by other authors (Ruttens et al., 2006) who investigated the leaching of metals in relation to the application of metal-immobilizing soil amendments, thus suggesting that the destruction of soil aggregates has an effect on (de)sorption processes and oxidation of soil functional groups alters the structure of compounds and their ability to bind metals, resulting in increased metal mobility.

5.2 The Physiological Characteristics of *M. malabathricum* L. in Acidic Conditions

The relative chlorophyll content of plants grown in PK biochar treatment showed the highest value followed by treatment with FW compost and BL. The chlorophyll content is one of the most vital biochemical indicators (Liu et al., 2014). According to Uysal (2018), the synthesis of chlorophyll relies on the presence of nutrients such as nitrogen and potassium. It was evident from Figure 4.6 that the application of PK biochar resulted in the highest chlorophyll content compared to other treatments. The outcome of these research findings is consistent with those of Halim et al. (2018) who also reported a significant increase in relative chlorophyll content in plants treated with biochar. Increased soil acidity is regarded as the main factor that controls the chlorophyll content in the leaves of *M. malabathricum* L. The application of PK biochar and FW compost increase the soil pH and significantly increase nutrient availability to improve the soil macronutrient particularly total N, available P and K. Nitrogen is one of the main components for the synthesis of chlorophyll and photosynthetic enzymes required in the process of nitrogen metabolism such as nitrogen reductase, glutamate synthase, glutamine synthetase and glutamate dehydrogenase (Tischner, 2000; Yang et al., 2021).

According to a study conducted by Teshome et al. (2017), it was demonstrated that a soil ameliorant could reduce the characteristics of acidic soil effect and increase plant development. The results from this research demonstrate that the soil pH increased for all soil treated with amendments compared to the control. This result implied that the higher soil pH would maximize the photosynthetic efficiency (Gentili et al., 2018). Moreover, Dighton et al. (2014) also revealed that the soil pH would affect the accessibility and uptake of micro-nutrient such as magnesium which is implicated in photosynthetic efficiency. The stomatal conductance and transpiration rates showed similar trends whereby the combined compost and lime (CL) and lime (L) treatments showed the highest readings in the final planting period for both parameters. The stomatal conductance and transpiration rate increased with sufficient soil water available for plant growth (Yadav et al., 2019). Compared to the controls, the exogenous application of amendments increased the photosynthetic parameters in the plants. The decline in photosynthetic characteristics of control (Q) plants could be caused by the contamination from heavy metals in the chloroplast structures, which decreased the efficacy of photosynthetic activity systems (Rizwan et al., 2016a, Rizwan et al., 2016b). This lowest photosynthetic rate and stomatal conductance are caused by stomatal closure. The application of amendments helps to restrict stomatal closure and improve the CO_2 assimilation required for the photosynthesis process (Arancon et al., 2004). Acidic soil conditions also caused damage to photosynthetic pigments and decreasing in stomatal conductance due to ion toxicity in the cytosol (James et al., 2006). ABA, which has been identified as a key regulator of stomatal mobility, is secreted at higher levels in response to acidic stress, thus triggering changes in guard cells' ion homeostasis, which then causes the stomata to close (Daszkowska-Golec et al., 2013). These guard cells generate ROS such as H_2O_2 (Zhang et al., 2001) that play a role when exposed to stress conditions.

A strong positive correlation between stomatal conductance, transpiration rate and calcium were observed in this study. This finding offers additional evidence supporting to the widely held belief that a relationship exists between stomatal conductance and transpiration rate. Significantly, this study illustrates the role of calcium in augmenting both photosynthetic efficiency and stomatal conductance in plants. This conclusion is consistent with the results reported by Sitko et al. (2019), who also demonstrated that the addition of calcium enhances the efficiency of photosynthesis and stomatal conductance in plants exposed to acid precipitation and heat stress. The regulation of stomatal opening and closing relies on the alterations in the internal pressure of the guard cells; hence increased turgor pressure resulting from elevated solute accumulation thus promotes the aperture of pores (Hosy et al., 2003). Moreover, the impact on plant growth is significant when there is a lack of crucial macronutrients, such as phosphorus, magnesium, potassium, or calcium, as elucidated by de Souza Osório et al. (2020). The presence of calcium was found to exhibit a significant positive correlation with soil pH and electrical conductivity. This finding aligns with a similar observation made by Behera et al. (2015) in acid soil in India, where it was noted that soil pH and EC content were also positively and significantly correlated with calcium implying that this element able to enhance growth and photosynthesis through improved soil properties.

Within the framework of our study, the correlation established between FW compost treatments with root volume and diameter demonstrates a complex connection that has implications for multiple aspects of plant physiology. The root volume and diameter are crucial factors that influence the intake of nutrients and the absorption of water (Yang et al., 2004). These factors are closely linked to the rate of transpiration and the availability of important macronutrients such as phosphorus (P). The possibility of increased transpiration rates through enhanced water absorption capacities can be attributed to the

promotion of augmented root development facilitated by FW compost treatments. Consequently, this phenomenon has the potential to enhance the transportation of essential minerals, such as phosphorus, across the root structure, subsequently impacting its accessibility for various metabolic activities (Fidelibus et al., 2001). Moreover, there is a suggestion that sucrose may have a role in enhancing the response of the root system to auxin under conditions of phosphorus deprivation (Hammond et al., 2008). In addition, it becomes evident that there is a complex relationship between the morphology of plant roots and the rate of photosynthesis. A well-developed root system can enhance the availability of water and nutrients to the plant, thereby promoting optimal photosynthetic activity. A study by Wissuwa et al. (2005) shows that the decrease in shoot biomass observed in the presence of low phosphorus (P) levels can be attributed to a decline in net photosynthesis. Therefore, it is possible that tolerance to low P might be related to efficient ROS scavenging enzymes, which enabled the tolerant genotype to overcome the stress and support growth (Veronica et al., 2017). Therefore, the interaction of these interrelated factors highlights the complex relationship between FW compost treatments, root structure, transpiration rate, phosphorus availability, and photosynthesis rate, revealing a comprehensive view for comprehending and enhancing plant responses to soil amendments in order to promote plant growth.

5.3 Effects of Different Soil Amendment Approaches on Plant Cellular Antioxidants and Oxidative Stress Indicators.

5.3.1 Oxidative Stress Indicators

The result in oxidative stress indicators analysis showed a similar finding with free proline content whereby the H_2O_2 level in the control treatment was significantly higher compared to the plant treated for PK biochar and FW compost. The *M. malabathricum* L.

grown in an acidic soil condition showed a significant accumulation of H_2O_2 level. Both FW compost and PK biochar amendments in acidic soil played a significant role in lowering the H_2O_2 level. The application of FW compost and PK biochar significantly ($p \leq 0.05$) improved the *in vivo* H_2O_2 levels in the plants of both amendments. This finding shows that an application of FW compost and PK biochar as soil amendments reduced H_2O_2 levels in *M. malabathricum* L. grown under acidic stress by enhancing the oxidative defense system. These results are supported by Kul et al. (2021) in which the group reported reduced H_2O_2 levels of salt-stressed *Phaseolus vulgaris* L. when treated with biochar.

5.3.2 Enzymatic Antioxidants.

In this study, organic amendments have been shown to improve the activity of enzymes involved in antioxidant activity. *M. malabathricum* L. plants that were exposed to an acidic soil condition (as environmental stress) can incur higher endogenous production of ROS. Plants have developed a variety of mechanisms including the induction of antioxidant enzymes such as SOD, CAT and glutathione peroxidase (GPX) to scavenge ROS and protect themselves from damage (O'Brien et al., 2012). In the present work, the activity of SOD and APX enzymes in the leaves, stem and root of the plants was higher when supplemented with FW compost followed by PK biochar. However, CAT was an exception to this pattern. A similar finding was observed in the study by de Oliveira et al. (2017) where *Passiflora edulis* S. cultivation with the application of biofertilizer resulted in increased activity of antioxidant enzymes, such as SOD and APX, whilst displaying reduced CAT levels. The results suggest that SOD and APX activities are the main antioxidants defense mechanisms induced by these two amendments. A higher APX activity is probably associated with the higher ascorbic acid content in the extract of all parts. SOD scavenges radical superoxide by catalyzing its conversion to H_2O_2, which was

subsequently neutralized by CAT or APX. The oxidative process caused damage to the mitochondrial protein, hence the production of ROS is augmented (Dinakar et al., 2010). Studies have shown that interventions aimed at improving the cellular antioxidant defenses of plants can protect them against various stresses and diseases. There is still much to learn about the role of the plant cellular antioxidant defense system in health and disease, but it is clear that it is an important part of plant health and resilience.

In response to environmental stress, hormones such as abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and ethylene modulate the expression and activity of these enzymes (Ku et al., 2018). These hormones function as signaling molecules, coordinating the plant's defense mechanisms and adaptations to acidic soil conditions. In addition, Biochar decreased CAT and, H_2O_2 levels in *M. malabathricum* L. grown under acidic stress conditions, as shown in Tables 4.8 and 4.10, respectively. Introducing organic amendments has the potential to mitigate the detrimental effects of acidic stress by regulating the activity of antioxidant enzymes in crops (Tartoura et al., 2014). Understanding the hormonal and environmental regulation of enzymatic antioxidants is crucial for elucidating the complex network of factors that contribute to plant protection against acidic soil-induced damage and devising strategies to improve acid stress tolerance in plants. Although significant progress has been made in understanding the function of enzymatic antioxidants in protecting plants from abiotic stress conditions, there are still several unexplored areas and research gaps. Exploring the role of other enzymatic antioxidants and non-enzymatic antioxidants in acid stress tolerance will increase our knowledge of the antioxidant defense system in plants as a whole.

5.3.3 Non-Enzymatic Antioxidants

The adverse impact of plants grown under stress conditions, such as acidic soil, is

attributed to the destruction of chloroplasts, the organelles responsible for photosynthesis, and a decrease in enzyme activity involved in photosynthetic pigment production (Murkute et al., 2006). This study demonstrated that the application of FW compost and PK biochar resulted in an increase in photosynthetic pigments content in *M. malabathricum* L., indicating the potential mitigation of the negative effects of acidic soil conditions on the plants treated with both amendments. The photosynthetic efficiency is reduced by ROS generated within the cellular compartments such as chloroplast and peroxisomes (Sachdev et al., 2021). Excessive production of ROS is induced by unfavorable environmental conditions, causing a decrease in stomatal conductance, CO_2 assimilation, and/or the formation of excited triplet chlorophyll (^{3}Chl*) that disturbs the photosynthetic ETC and leads to photo-oxidation (Shakirova et al., 2016).

In line with these findings, the current study observed that the application of FW compost significantly increased carotenoid release in the leaves and enhanced the chlorophyll content. This observation was supported by strong positive correlations between carotenoid content and chlorophyll a ($r=0.984$, $p \leq 0.01$), chlorophyll b ($r=0.946$, $p \leq 0.01$) and total chlorophyll ($r=0.979$, $p \leq 0.01$) (Table 4.6). Similar results were reported by Neagoe et al. (2005) who found that the highest chlorophyll and carotenoid contents were observed in rye (*Secale cereale* L.) and lupine (*Lupinus angustifolius* L.) plants grown with municipal compost under acid mine drainage conditions. These findings highlight the potential of compost application to improve chlorophyll and carotenoid production in plants exposed to acidic soil conditions, thus suggesting a beneficial role in mitigating the negative effects of acid stress. The application of FW compost significantly improved the leaf enzymatic activities, which can alleviate the ROS stress caused by acidification (Jaspers et al., 2010; Sari et al., 2018). Hence, this led to chlorophyll and carotenoid content increase with the application of FW compost. The

increased carotenoid content in the leaves may also be associated with the increased nitrogen availability in FW compost and PK biochar amendment with improved water balance (Chaves et al., 2002). This is parallel to the results in this study, whereby soil N had a significant positive correlation with total chlorophyll and carotenoid with r=0.851 and r=0.914, respectively at a p-values of less than 0.01 (Table 4.6).

The *M. malabathricum* L. plants grown in an acidic soil condition showed a significant accumulation of proline level. Proline, renowned for its role in stabilizing cellular structures, enzymes and scavenging ROS, also helps to maintain redox balance during adverse situations and acts as an osmoprotectant (Meena et al., 2019). Although proline level as a non-enzymatic defense system is lower than control treatment, other enzymatic antioxidant such as SOD, CAT and APX were significantly induced with FW compost treament, thus in synergy, will help in scavenging ROS. These results are in agreement with Kul et al. (2021) in which they reported reduced proline levels in salt-stressed *Phaseolus vulgaris* L., when treated with biochar. Both amendments provided the development of anti-stress reaction by maintaining the proline accumulation at all parts of the plant (Sakhabutdinova et al., 2003).

It is well documented that when plants are under stress, the concentration of proline, which accounts for up to 80% of the total amino acid pool, rises up to 100 times above the baseline level in many plants (Dolatabadian et al., 2009). Proline has been suggested to function as a compatible solute that modifies osmotic potential in the cytoplasm in addition to its involvement in radical scavenging activities and protecting enzymes (Meena et al., 2019; Rejeb et al., 2014). In the current study, the acidic stress exerted onto the control plants increased the leaf proline content, which might have contributed to osmotic adjustment and allowed the plants to maintain turgor pressure, thereby enabling

them to adapt to soil acidity. It is now known that the application of FW compost and PK biochar as soil amendments in acidic soil may overcome the stress responses by enhancing the activities of antioxidant enzymes, as shown by the increase in SOD and APX activities observed in this study.

The content of ascorbic acid (AsA) in all parts of the plant is higher in plants supplemented with FW compost followed by PK biochar. AsA engages in respiration and photosynthesis as a non-enzymatic antioxidant in metabolic processes (Franceschi et al., 2002). AsA also can provide membrane protection by scavenging the $^{1}O_2$, O_2^{1-} and OH^{1} and regenerating tocopherol from tocopheroxym radical (Thomas et al., 1992). The supplementation of these two amendments into acidic soil resulted in an increase in AsA value, thus proving that both functions to minimize the damage caused by the oxidative stress. When FW compost and PK biochar were applied to *M. malabathricum* L. plants grown on an acidic soil, the pool of AsA was restored as compared to the control (Q) plants that were grown under acidic stress. Similar results have also been observed by Ramzani et al. (2016), who discovered that biochar treatment yielded in an increment of AsA content in rice grown in soils contaminated with nickel.

5.3.4 Secondary Metabolite Production

The qualitative screening of Melastomataceae leaf extracts has been widely reported (Ahmed et al., 2019; Danladi et al., 2015a; Danladi et al., 2015b). However, to date, no available studies have reported on the biochemical properties of various parts of *M. malabathricum* L. supplemented with FW compost and PK biochar. The results revealed the presence of flavonoids and phenols (Table 4.17). A similar phytochemical was also reported to be present in the leaves of *M. malabathricum* L. (Sari et al., 2018; Zakaria et al., 2006). Meanwhile, tannins were observed to be present in the leaf and root

extracts, whilst alkaloid was only present in the leaf extract.

Organic amendments were found to increase the production of the TAC, TPC and TFC in *M. malabathricum* L. leaves grown in acidic soil conditions. The leaf methanolic extracts obtained from FW compost contained the highest TAC followed by PK biochar and control plants (Table 4.18). A similar trend was observed in the root methanolic extracts. Both TPC and TFC of the leaf and root extracts exhibited a similar trend with the highest TPC was recorded in PK biochar, followed by FW compost and the lowest was exhibited by the control plants. On the contrary, Yusof et al. (2018) reported that with the lower TAC, TPC and TFC values, organic amendment (vermicompost) had no significant effect on the expression of bioactive compounds. They argued that Al toxicity could result in a rise in ROS, which could either boost or decrease antioxidant ROS-scavenging activities. However, such a condition was not observed in the current study. This shows that when *M. malabathricum* L. plants were supplemented with organic amendments, the plant or plant cells experienced elicitation or enhanced biosynthesis of secondary metabolites due to the addition of trace amounts of elicitors (Namdeo, 2007). Elicitors act to stimulate a response in plants from biotic or abiotic sources, resulting in increased synthesis and accumulation of secondary metabolites or the induction of novel secondary metabolites (Naik et al., 2016), as observed in this study with FW compost and PK biochar supplementations. A comparable finding was observed using date palm waste compost utilized in the study by Ghouili et al. (2022), where of the use of various elicitor compounds was found to induce the accumulation of salicylic acid (SA) and jasmonic acid (JA), while also increasing the expression of defense-related proteins, which produced proteins involved in the metabolic processes of phytohormone, DNA methylation, and secondary metabolites.

In addition, the increase in soil pH was observed to lead to higher anthocyanin accumulation in the *M. malabathricum* L. plants, particularly at pH values ranging from 5.25-6.25 (Chan et al., 2010). This was achieved with the supplementation of organic amendments, especially FW compost, and this response was annotated by a high positive correlation between TAC with pH ($r=0.869$, $p \leq 0.01$) (Table 4.6). Anthocyanin has been reported to help reduce damage caused by free radical activity (Murapa et al., 2012). Increased anthocyanins were associated with increased antioxidant capacities, which may facilitate the decrease of active oxygen species' excited state (Wang et al., 2003).

Accumulation of flavonoids can be induced by various environmental factors (Stefanelli et al., 2010). Manipulation of environmental conditions such as light intensity and nutrient availability is a significant approach for stimulating secondary plant metabolite production, particularly for plants under an intensive management system (Deng et al., 2012). Higher accumulation of rutin in PK biochar followed by FW compost indicated that these amendments were able to protect *M. malabathricum* L. against damage by ROS (Li et al., 2022) caused by acidic stress conditions, by upregulating rutin synthesis. Similar results have been reported in drought-stressed *Fagopyrum esculentum* treated with vermicompost and mycorrhizal fungi (Mohammadi et al., 2022).

5.3.5 Induction of Metabolites in Leaves and Roots of *M. malabathricum* L. Grown Under Acidic Conditions

Numerous metabolites with antioxidant potential are induced by FW compost and PK biochar. These treatments have shown to elicit notable responses in terms of metabolite production. Among these induced metabolites, scopoline and arjunolic acid have emerged as significant compounds. An intriguing observation is that both metabolites can be found in the leaves of all treatments, suggesting their fundamental roles in the antioxidant

defense mechanisms in the studied sample. The scopoline content may mitigate the negative effects of inadequate K uptake, such as impaired stomatal regulation, excessive vapor loss and stunted plant growth (Hasanuzzaman et al., 2018). Additionally, arjunolic acid, belonging to the triterpenoid group, indicates that it plays an important role in activating the plant defense response. Meanwhile, the detection of γ-Tocotrienol in control plants indicates its crucial role in protecting plants from abiotic stressors. γ-Tocotrienol is highly effective at preventing lipid peroxidation and more effective at scavenging ROS (Xiang et al., 2019).

Some potential compounds were found to be associated with ROS scavenging and pathogen defense, for example in a research by Döll et al. (2018), *Arabidopsis thaliana* grown under abiotic stress showed accumulation of a coumarin, i.e scopoline. Coumarins are an important group of natural compounds that provide antimicrobial and antioxidative activities for plants and serve as a defense mechanism against pathogen attack and abiotic stresses (Baillieul et al., 2003). Large quantities of coumarins, such as scopolin, scopoletin and ayapin are thought to play an essential role in a plant's response to fungal infection and subsequent stresses (Ahn et al., 2010). Apart from its direct function as a fungitoxic agent, scopoletin can serve as a substrate for peroxidase to polymerize scopoletin in the presence of H_2O_2 (Reigh et al., 1973) and protect cells from the oxidative damage caused by microbial infection.

Arjunolic acid is another compound with a role in preventing damage (Sumitra et al., 2001). Arjunolic acid is a triterpenoid saponin that is well-known for various biological functions (Ghosh et al., 2013). Arjunolic acid is produced in response to environmental stresses such as drought, heat and pathogen attack, and it functions as a protective agent against injury. It has been demonstrated that arjunolic acid protects plant cells from

oxidative stress by decreasing the production of ROS and promoting the activity of antioxidant enzymes (Hemalatha et al., 2010). Under stress conditions such as acidic soil, *M. malabathricum* L. produces more ROS, which can cause cell damage and even mortality. In response to acidic stress, this can facilitate the production of more protective compounds such as flavonoids and phenolics, which have demonstrated antioxidant and anti-inflammatory properties. These compounds further protect the plant against stress-induced damage. Arjunolic acid reduces the accumulation of ROS, thereby protecting the cell from oxidative injury.

γ-Tocotrienol, a type of vitamin E that is present in numerous plants, was detected in the control plants. Recent research has shown that γ-Tocotrienol plays a crucial function in protecting plants from abiotic stressors such as high temperature and drought (Gudys et al., 2018; Réblová, 2006). When plants are subjected to stressful conditions, such as acidic soil, their cell membranes become more permeable, which can result in cell damage and mortality. It has been demonstrated that γ-Tocotrienol aids in the stabilization of cell membranes under conditions of stress by decreasing membrane fluidity and preventing lipid peroxidation (Phang et al., 2023). This contributes to the maintenance of cellular integrity and function, even under stressful conditions. Tocopherols have been shown to profuse in plastoglobules of leaves, whereby there is evidence that plastoglobules contain high concentrations of carotenoids (mainly phytoene and pro-vitamin A β-carotene) as well as other nutritionally significant isoprenoids such as tocopherols (vitamin E) and phylloquinone (vitamin K1) (Morelli et al., 2023). Plastoglobules are thylakoid protrusions composed of lipophilic constituents such as triacylglycerols, quinones, chlorophyll, carotenoids, as well as monogalactosyldiacylglycerol and digalactosyldiacylglycerol, which increase in number during senescence and oxidative stress (Munné-Bosch et al., 2004). Free phytol from chlorophyll degradation may be

recovered directly for tocopherol biosynthesis (Dörmann, 2007), which is required for antioxidant protection under stress conditions.

5.3.6 Distribution of Auxin in Shoot, Stem and Root of *M. malabathricum* L.

The use of soil amendments is crucial for improving the growth performance of plants in acidic soils. Indole-3-acetic acid (IAA) is a significant signaling molecule and plays a crucial role in stress tolerance (Shani et al., 2017). In addition, auxin has the ability to mitigate the effects of various stresses, such as salinity, drought, chilling, heat, and heavy metals (Jing et al., 2023). In this study, the levels of auxin IAA, IBA, and IPA were below the limit of quantification (LOQ), making them undetectable (Table 4.8).

A study by Xu et al. (2013) reported that in the root apex, moderate water stress increases abscisic acid (ABA) accumulation and auxin transport. In order for the root tip to adapt to moderate water stress, the transported auxin activates the plasma membrane H^+ -ATPase and causes it to release more protons. Under moderate water stress, the root tip's proton secretion is crucial for maintaining or encouraging primary root elongation and root hair development. IAA levels were drastically reduced under water stress in both roots and shoots (Ghosh et al., 2019). At harvest, auxin content dropped significantly and was maintained at a low level (Kender et al., 1970).

Numerous plants can interconvert IBA and IAA, which means that their conjugates may have similar functions in the plant such as inactivating IAA or acting directly as an auxin (Strader et al., 2010). The presence of IBA in high concentrations in maize leaves and kernels suggests that this auxin is widely distributed throughout the plant (Epstein et al., 1989). In addition, there is early evidence by Feito et al. (1996) that shows the concentrations of IAA and ABA are the lowest in the rooting systems with the highest levels of cytokinins, primarily zeatin and its riboside. Auxin's role in plant growth, development and more recently in stress response may be mediated by ROS signaling, which has been observed in a number of studies including the significance in auxin and ROS regulation of plant growth, development and stress tolerance such as temperature

(Krishnamurthy et al., 2013). The use of HPLC to quantify endogenous indole-3-propionic acid (IPA) by Hashtroudi et al. (2013b) also demonstrated that IPA was unable to be detected in their samples, which is in line with this current study.

There is limited supporting evidence in the literature regarding the correlative studies of acidic stress and endogenous hormone including IBA and IPA. There has been in the literature for many years that the low concentration of hormones in plants is one of the main issues in measuring endogenous auxins (Hashtroudi et al., 2013a). Incorporating HPLC with a fluorescence detector (FLD) followed by LCMS-MS may enhance the determination of these hormones and validate the presence of these hormones, thus shifting the focus of the current methods of research in a different direction. Stress such as soil acidity can disrupt the hormonal balance of plants, thus resulting in modifications in auxin balance and regulation (Pavlović et al., 2018). Consequently, the concentration of auxin in plant tissues may decrease, thus making it more challenging to detect and quantify using HPLC. Under adverse condition, auxin biosynthesis may be downregulated, thus reducing its production and availability in plant tissues (Bielach et al., 2017). In addition, stress can disrupt the transport mechanisms responsible for the movement of auxin within the plant. Disruptions in auxin transport can lead to altered distribution patterns and uneven accumulation of auxin in different plant tissues (Sharma et al., 2015), which further complicates its detection and quantification below the LOQ.

CHAPTER 6: CONCLUSION

Conclusions from this research support the widely held belief that plants are continuously subjected to abiotic stresses. An important factor in sustaining soil fertility is acid stress, which elicits a variety of plant responses. The effects of abiotic stress such as the exposure to soil acidity on plant growth include nutrient assimilation, osmotic adjustment and photosynthetic activity limitations. In addition, this stress induces alterations in phytochemical content, ROS levels, phytohormones and metabolites as part of the tolerance responses of plants toward unfavorable environmental conditions.

The preceding results showed that the utilization of PK biochar significantly improved the growth (chapter 5.1.1.) and the physiological characteristics (chapter 5.2) of *M. malabathricum* L. compared to the control (plants grown on acidic soil without amendment). The application of PK biochar onto acidic soil resulted in the production of taller plants with larger stem diameters, higher LAI, increased root length and root length density, and improved relative chlorophyll content and rate of photosynthesis.

The results of soil analysis showed that the application of PK biochar produced comparable results with FW compost (Figure 6.1). Both organic amendments were able to retain nutrient content in the soil, improved soil pH and water holding capacity, as indicated by the increase in the stomatal conductance. Nonetheless, some of the plants' nutrient absorption was lower compared to the usage of lime (as a conventional method) to improve soil acidity. Accordingly, it could be concluded that neither lime nor organic amendment could provide the optimal concentration of nutrients required by acidic tolerant plants when used as the sole source of nutrients to support the growth of plants subjected to acidic stress. Therefore, more investigations must be conducted to determine the optimal ratio of organic and other fertilizers required for optimum plant growth

performance.

Addressing the oxidative stress indicators, this study demonstrated that the treatments with FW compost, followed by PK biochar significantly enhanced the oxidative defense system of the plant. For example, both FW compost and PK biochar treatments significantly improved the activities of enzymatic antioxidants (such as SOD and APX) as well as other non-enzymatic antioxidants (chlorophyll, carotenoid and ascorbic acid contents). These demonstrate that the supplementation of organic amendment onto acidic soil resulted in increased induction of phytoconstituents to ensure the plant's survival. Metabolite profiling analysis showed that the supplementation of FW compost and PK biochar induced the production of 6-Hydroxyluteolin 5-rhamnoside, undulatone and unknown compound $C_{33}H_{51}N_{15}O_5$ in the plant leaves, which possessed beneficial antioxidant properties and demonstrated potential implications in a variety of physiological processes, including stress tolerance, defense against pathogens, and plant health. In addition, these secondary metabolites have been linked to enhancing plant resilience and adapting to varying environmental conditions, making them essential components of plant survival strategies. On the other hand, the supplementation of these amendments (FW compost and PK biochar) induced the synthesis of 2-[4-(3-Hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol 1-glucoside and 2 unknown compounds ($C_{15}H_8O_8$ and $C_{30}H_{42}O_3$) in the plant roots, which function to protect roots from oxidative stress by scavenging reactive oxygen species and maintaining redox homeostasis. In addition, these secondary metabolites play a crucial role in enhancing nutrient absorption and promoting root growth and development, thereby contributing to the overall vitality and productivity of the plant, thus improving nutrient uptake compared to that observed in control (Q) plants. These observations thus highlight the immense potential of these soil amendments (FW compost and PK biochar) in mitigating acidic

stress in plants, by modulating the synthesis antioxidant enzymes and secondary metabolites. However, these metabolites profile in plants has received limited attention; therefore, additional research is required to determine the reasons for the presence of these metabolites observed in this study.

The specific mechanisms underlying the responses of plants to abiotic acid stress and the effects of FW compost and PK biochar amendments on plant growth and stress tolerance require further investigation. Understanding these mechanisms will aid in the development of effective strategies for mitigating the negative effects of acid stress on plants, thereby promoting sustainable soil fertility and agricultural practices.

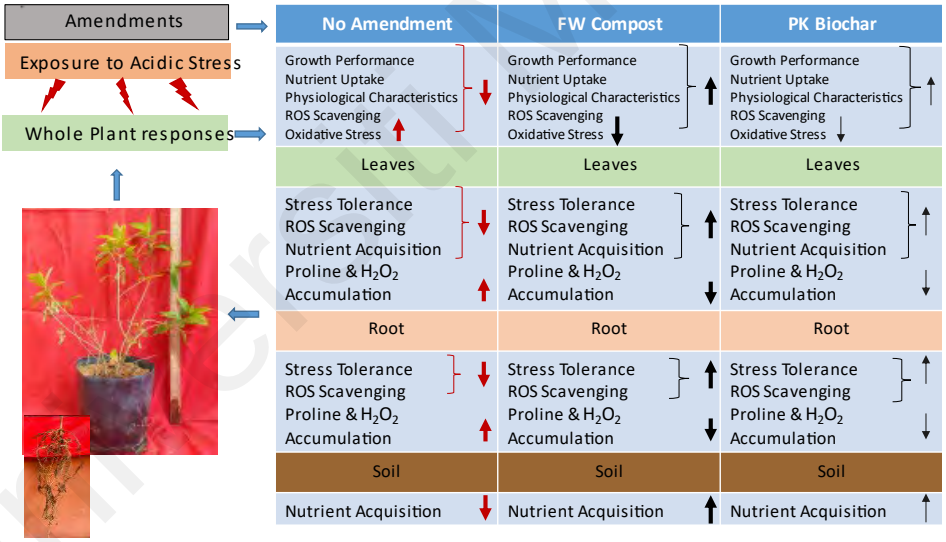


Figure 6.1: Overall summary from the research findings

6.1 Suggestions for Future Research

This study establishes the groundwork for future research on the use of FW compost and PK biochar in acidic soil. It is crucial to evaluate the potential of organic amendments to aid in the stress response of various plant parts through research involving their use. Other abiotic and biotic stresses on plants should be investigated in order to gain a deeper understanding of stress mitigation and plant growth performance. To advance the

investigation, it is suggested that a variety of omics platforms, including proteomics, transcriptomics and genomics, be explored. These platforms provide important insight into protein functions, gene expression patterns and genetic variations, allowing for a comprehensive understanding of plant biology. In addition, the incorporation of nanotechnology as a bioindicator of plant stress in future research hold a great promise. Nanotechnology provides precise and sensitive instruments for monitoring molecular level stress related changes.

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